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Agriculture Development Fund

FINAL REPORT

19990266

OPTIMIZATION OF A BIOLOGICAL BLACKFLY LARVICIDE IN SASKATCHEWAN RIVERS

Funded by
The Agriculture Development Fund

November 2001

Prepared by: District 32, ADD Board

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102-02307/

OPTIMIZATION
OF A
BIOLOGICAL LARVICIDE
FOR
BLACKFLIES (DIPTERA: SIMULIIDAE)
IN THE
SASKATCHEWAN RIVER

Final Report

Submitted to:

The Agriculture Development Fund
Project No. 1999 0266

Submitted by:

Prince Albert Agriculture Development and Diversification Board
District #32
Prince Albert, Saskatchewan

September 2001

AGRICULTURE DEVELOPMENT FUND
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Executive Summary

In an effort to reduce the economic impact to livestock producers from damaging outbreaks of blackflies (Diptera: Simuliidae), a Saskatchewan blackfly control program has targeted blackfly larval habitat in the Saskatchewan Rivers for pesticide applications. Four efficacy trials using the blackfly larvicide *Bacillus thuringiensis* variety *israelensis* (*B.t.i.*) were conducted on the North Saskatchewan River, 3 during the spring of 2000 and the 4th treatment was completed in the June 2001. These trials targeted the seasonal population of *Simulium luggeri* (Nicholson and Mickel) larvae in habitat downstream of the City of Prince Albert, Saskatchewan. *B.t.i.* dosages varied with respect to concentration within the river discharge at 2.5 parts per million (ppm) water / 15 min., 2.0 ppm / 15 min., 1.5 ppm / 15 min., and 1.0 ppm / 15 min. The methods for *B.t.i.* application to the treatment area was consistent for each trial, which involved a twenty foot jet boat and spray equipment which released the *B.t.i.* dosage as it navigated in a 'zig zag' pattern downstream.

Measures of larval density were observed using artificial substrates over a 33km area for each trial. Sampling locations were from an upstream site above the treatment area which served as a reference, and 11 sites positioned equidistantly in mainstream locations throughout the application zone. Artificial substrates were placed 1wk prior to *B.t.i.* applications to allow colonization by blackfly larvae. An index of mortality was calculated by comparing pre-treatment samples collected 2 - 4hrs prior to *B.t.i.* application with post treatment samples collected 48 hrs after application of *B.t.i.*. An estimate of the proportion of blackfly larvae at drift during time of treatment was used in the expression of mortality.

The results of each trial were compared using a Page Test of Ordered Alternatives and found to be significantly different ($Z_L = 5.91$, $N = 33$, $k = 4$, $P < 0.00003$). Further multiple comparisons determined median mortality (θ) was significantly different among 2 groups: $\theta_{2.5\ ppm}$ and $\theta_{2.0\ ppm} \neq \theta_{1.5\ ppm}$ and $\theta_{1.0\ ppm}$, $P < 0.05$ in a one tailed test. Thus, dosages calculated from *B.t.i.* concentrations of 2.5 and 2.0 parts per million result in higher larval mortality than dosages calculated at 1.5 ppm and 1.0 ppm (ΔR ranged from 43 - 59 $> R_{critical} = 25$, $P < 0.05$). Conversely, differences in larval mortality could not be determined between treatments at 2.5 ppm and 2.0 ppm nor between treatments of 1.5 ppm and 1.0 ppm ($\Delta R = 6 < R_{critical} = 17$, $P > 0.05$). These results should provide a more objective framework for pest managers when determining *B.t.i.* treatment dosages to effect a desired level of mortality.

ACKNOWLEDGMENTS

This project was administered by the Prince Albert Agriculture Development and Diversification (A.D.D.) Board, District #32. The transition from synthetic chemical larvicides to a biological control over concern of the Saskatchewan River ecology has been a challenge for the blackfly program. Delivery of an effective blackfly control program within a limited operating budget has been further confounded by utilizing a new biological agent with little application technology available in the literature. This effort was intended to describe the precise effect of *Bacillus thuringiensis* variety *israelensis* upon Saskatchewan blackfly larvae for increased cost effectiveness.

Field personnel and larvicide for this project were funded through research grants from the Agriculture Development Fund (ADF) and the Canadian Adaptation and Rural Development-Saskatchewan (CARDS). The Prince Albert A.D.D. Board gratefully acknowledge the financial contribution to this project.

The field trials were supervised by Mr. S. Lipsit and Mr. S. Meckelborg who also collaborated in the writing of this manuscript. Many thanks to Mr. D. Harbidge and Mr. R. Meschishnick for their technical assistance during the study. This project would not have been possible without the dedication of previous blackfly investigators. We acknowledge the work of Dr. F.J.H. Fredeen, Dr. P. Mason, and Dr. E. Whiting who maintained the highest scientific standards for applied entomology in Saskatchewan. It is hoped this is a continuation of their work.

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1. INTRODUCTION

1.1 General Introduction

The Saskatchewan River continues to be a source of an adult blackfly population with severe economic impacts for regional livestock producers. Historically, periodic outbreaks of *Simulium arcticum* complex (Malloch) which emerged from the Saskatchewan River system were responsible for the death of hundreds of cattle due to acute toxemia (simuliotoxicosis) from biting females (Fredeen 1951, Rempel and Arr son 1947). Today the dominant pest species in terms of economic importance is *Simulium luggeri* (Nicholson and Mickel) which has increased in abundance concurrent with ecological changes in the Saskatchewan River profile. Fredeen (1977) argues construction of hydroelectric facilities and associated reservoirs on the South Saskatchewan River and the headwaters of the North Saskatchewan River have all but eliminated the univoltine pest *S. arcticum*. Slower, less turbid conditions and increased primary production have provided more suitable conditions for the multivoltine species *S. luggeri*, *Simulium meridionale* (Riley), and *Simulium vittatum* complex Zetterstedt (Fredeen 1977, Jarvis 1987). *S. vittatum* although mammalophilic has minimal economic impact to the region and *S. meridionale* is ornithophilic and is of little economic importance. Females of *S. luggeri* are mammalophilic, attacking man and cattle for a blood meal as nutrition for egg development. Although cattle mortality is rare from *S. luggeri* outbreaks, their multivoltine nature can produce chronic infestations which may increase exponentially from the first generation in June until late September. The economic impact for Saskatchewan cattle producers within a 5,700 km^2 area has been estimated to exceed $3,000,000.^{00}$ (Fredeen 1985). Losses are incurred primarily from unrealized weight gains, reduced milk production, missed estrus cycles, and debilitated animals. The attendant costs of increased labor and veterinary services are difficult to assess (Fredeen 1979, 1985a).

Organized control efforts with DDT applications to the Saskatchewan River system began in 1948 and continued periodically until 1967 to control the pest species *S. arcticum* complex (Fredeen 1962, Fredeen *et al.* 1953, Fredeen *et al.* 1975). The larval phase of *S. arcticum* populations were targeted because they were restricted to the river system whereas the adults

become widespread and unconstrained. With the recognition of the persistence of DDT in the environment and the bioaccumulation of its residues in food webs, pest managers relied on methoxychlor (1,1,1-trichloro-2,2-bis [*P*-methoxyphenyl] ethane) for control of *S. arcticum* and *S. luggeri* populations in the Saskatchewan River throughout the 1970's and 80's (Fredeen 1975, 1983). Methoxychlor is a chlorinated hydrocarbon similar to DDT as a blackfly larvicide but with a lower half life which reduces its persistence in the environment and food webs (Burdick *et al.* 1968, Fredeen *et al.* 1975). Methoxychlor was used as a blackfly larvicide with limited success. Chronic outbreaks occurred in east central Saskatchewan every year between 1975 and 1982, although Fredeen (1985a) reported methoxychlor reduced the potential severity of outbreaks. There is evidence to support the effectiveness of methoxychlor as a blackfly larvicide (Fredeen 1983), however, the catastrophic drift (downstream carry) of non-target organisms in the benthic assemblage is also well documented (Dosdall and Lehmkuhl 1989a, 1989b, Fredeen 1983, Muirhead-Thompson 1987, Wallace and Hynes 1975). Methoxychlor remains a registered blackfly larvicide under the Pesticide Products Act (Canada) but with restricted use due to concerns of long term ecological health, particularly with fish and benthic macroinvertebrates (Gardner and Bailey 1974). Saskatchewan Environment and Resource Management will no longer permit the use of organochlorine or organophosphate pesticide products in the Saskatchewan River system due to non target concerns in a valued fishery (personal communication).

In 1991 Saskatchewan blackfly control efforts turned to *Bacillus thuringiensis* variety *israelensis* (*B.t.i.*) as a biological alternative to synthetic chemical larvicide. *B.t.i.* is a naturally occurring entomopathic bacteria which has been batch cultured as a registered pesticide product. Efforts to control pest populations of *S. luggeri* using *B.t.i.* have been difficult to evaluate as to their success. Application technology stemmed from experience of pest managers with methoxychlor and other synthetic larvicide and it was quickly established that *B.t.i.* was not as effective when applied using conventional point injection techniques. Point injections relied upon the river discharge for downstream transport of the pesticide product, however, differences in the physical nature of *B.t.i.* formulations reduced downstream efficacy (Chalifour *et al.* 1990). Improvements in application technology increased the effectiveness of *B.t.i.* on blackfly larvae, up to 95 - 100% reduction in some trials (Lehmkuhl 1989), however dosage rates still required

refinement for cost effectiveness and to minimize non target impact. This study investigated the efficacy of 4 *B.t.i.* larvicide trials using TEKNAR HPD® upon blackfly larvae in the North Saskatchewan River as observed from changes in abundance on artificial substrates. In addition to efficacy observations associated with *B.t.i.* applications, annual trends in blackfly larval densities upon artificial substrates are described for the Saskatchewan River watershed within a delineated zone.

1.2 Objectives

The objectives of this study were to:

1. Evaluate the efficacy of four dosage rates of the blackfly larvicide *Bacillus thuringiensis* variety *israelensis* upon 33km of *S. luggeri* larval habitat in the North Saskatchewan River. Efficacy was determined using measures of larval densities upon artificial substrates before and after treatments. The function of *B.t.i.* dosage versus blackfly mortality was described in an effort to provide a meaningful framework for *B.t.i.* applicators and pest managers. The information is essential for improving cost effectiveness for the Saskatchewan blackfly control program and minimize pesticide residues in the environment, and;

2. Report observations of general blackfly larval population trends for the Saskatchewan River system as a result of larvicide application techniques. In addition to the efficacy monitoring, a system of long term artificial substrate monitors remained in place to measure trends in blackfly larval densities and population dynamics associated with control efforts. Since the dominant pest species *S. luggeri* is multivoltine, it is important that pest managers recognize the development of later generations and cohorts of pest populations. Recognizing development time and threshold densities is critical to timely larvicide applications and avoiding mass emergence of adult pest populations.

1.3 Natural History and Economic Importance of Blackflies

Blackflies (Diptera: Simuliidae) are a relatively small group of 'true flies' in the class Insecta. They occur worldwide except Antarctica and some deserts and islands where running water is not present (Crosskey 1990). They undergo four stages in their life history with the egg, larvae, and pupae occurring in aquatic environments and the adult, or imago, inhabiting terrestrial habitats. Larvae rely on flowing water due to a complex method of filter feeding from the water column. Labral, fan-like appendages are present on most genera which capture food particles while extended into the water current and are retracted to the mandibles for ingestion (Craig and Chance 1982). The constituents of immature blackfly diet are detritus, diatoms, bacteria, filamentous algae, and animal matter (Crosskey 1990). The size of particles ingested varies among species, larval instar and availability (Kurtak 1979). Blackflies are holometabolous insects which pupate within a woven cocoon produced from silk glands. Adults emerge using hydraulic pressure to split the cocoon and rise to the water surface on a bubble of air.

It is the adult phase of the life cycle which is of importance to human health and economy. Female blackflies may be autogenous and entirely lacking biting mouthparts, relying upon larval nutrition for the development of eggs. However, females of many species are anautogenous and are parasitic to vertebrate hosts and seek a blood meal for nutritional requirements of egg development. Females return to aquatic habitat and oviposit on semi-emergent vegetation, exposed rock, or release the eggs on the water surface. Blood seeking females may be a trivial nuisance in some instances, but the swarming, unrelenting types of infestation can have severe economic consequences in the fields of forestry, mining (Jamnback 1969), agriculture (Fredeen 1985a, Rocha *et al.* 1995), and tourism (Grey *et al.* 1996).

Toxins secreted by female blackflies during biting can cause acute toxemia (simuliotoxicosis) or anaphylaxis and, although death among humans is rare, livestock mortality is not uncommon (Mackerras and Mackerras 1948, Eckert et *al.* 1967 cited in Jarvis 1987, Fredeen 1979, 1985). More importantly, blackflies are vectors to some significant human and livestock diseases including the filaria *Onchocerca* which causes 'river blindness' in Africa and Latin America (reviewed by Boatin *et al.* 1997); leucocytozoonosis, a protozoan parasite of fowl which is often

fatal; and bovine onchocerciasis.

Some 23 species of blackfly are known to occur in Saskatchewan, with 15 species of blackfly larvae having been observed in the Saskatchewan River system (Adler and Mason 1996, Fredeen 1981, 1985b). There are 9 species identified from adult collections of blackflies from cattle pastures in east central Saskatchewan. Four morphologically distinct taxa *(S. luggeri, S. meridionale, S. vittatum,* and *S. venustum vercundum* complex) are the most frequently collected blackfly adults from pest populations harassing cattle and *S. luggeri* is recognized as the principal pest species (Fredeen 1985b, Mason and Kusters 1989a, 1989b). *S. luggeri* larvae have been observed to occur only in large rivers in Saskatchewan (Saskatchewan Rivers and Torch River) whereas other pest species described by Mason and Kusters (1989a) have been observed in large rivers and smaller streams (Adler and Mason 1997, Fredeen 1981).

Blackfly control efforts in Saskatchewan have focused on the North and South Saskatchewan River branches, the Saskatchewan River downstream of the confluence of the 2 branches, and the Torch River. The phenology and drift of species commonly found in the Saskatchewan Rivers, including *S. luggeri,* are well documented by Jarvis (1987). In 1984 three generations of *S. luggeri* were observed to emerge from the North Saskatchewan River (Jarvis 1987) and Fredeen (1981, 1985b) suggests as many as 5 may occur annually. Jarvis (1987) determined the larval development time for *S. luggeri* to be between 255-321 degree days and this calculation could conceivably allow 7 generations of *S. luggeri* to emerge from Saskatchewan Rivers. Sources agree the protracted time of the initial hatch of overwintering eggs in May, together with asynchronous development of larvae, make identification of discrete generations of *S. luggeri* nearly impossible (Fredeen 1981, 1985b, Jarvis 1987). The result is a virtually continuous emergence of *S. luggeri* adults increasing pest populations in the field exponentially from early June until late September annually. Protracted, chronic infestations typically overwhelm on-farm blackfly control measures such as repellants or adulticides and further support the argument for larvicide application strategies.

1.4 Blackfly Control Efforts

While the concept of integrated pest management (described by Johansen 1985) is often expounded by pest managers, the efforts to control insect pests still primarily rely on the use of synthetic chemical toxicants or repellants which target a particular life stage (Kachatourians 1986). In the case of blackfly pest populations, methods have paralleled or perhaps followed that of another biting fly, the mosquito. That is, targeting strategies have focused on the aquatic habitat where the population is relatively confined and the number of adult females can be effectively reduced before impact (Rogoff 1985). Becker and Margalit (1993) estimate the annual use of chemicals for mosquito and blackfly control worldwide exceed 50,000 tons of organochlorines, organophosphates and carbamates, with DDT still the most commonly used product. Advancement in chemical technology has improved in areas such as reduced impact and persistence of residues to non-target biota. Currently, large-scale control programs in Africa and Australia are relying largely on organophosphates such as Temephos (Ritchie 1994, Palmer *et al.* 1996a, 1996b) as well as Chlorphoxim (Yameogo *et al.* 1988), permethrin and carbosulfan (Leveque *et al.* 1988, Kurtak *et al.* 1989). Recent management strategies in response to insecticide resistance and nontarget impact have included the use of the microbial insecticide *Bacillus thuringiensis* variety *israelensis* (*B.t.i.*). Use of *B.t.i.* was on a rotational basis by the World Health Organization (WHO) in their onchocerciasis control operations (Becker and Margalit 1993) and small scale field trials in Australia (Ritchie 1994). The Saskatchewan Blackfly Control program began trials using *B.t.i.* in conjunction with methoxychlor in 1987 and finally discontinued the use of methoxychlor in 1991 (authors observations).

1.5 *Bacillus thuringiensis*

1.5.1 Isolation of *Bacillus thuringiensis*

Bacillus thuringiensis Berliner (*B.t.*) is a gram positive bacteria first isolated in 1905 from a diseased silkworm larvae, *Bombyx mori* (Fast 1974). The microbe was recovered again in 1910 from diseased flour moth larvae, *Anagasta kuehniella*, and named *Bacillus thuringiensis* by Berliner (Fast 1974). *B.t.* is a spore forming bacteria which was easily reared in the laboratory.

It was observed that larvae became symptomatic with the disease only from older, sporulated cultures and vegetative cultures could not induce pathogenesis (Fast 1974). Further investigation revealed pathogenicity to three orders of insect, Lepidoptera, Coleoptera and Diptera. Field trials using commercial formulations of *B.t.* began in the late 1920's and 1930's for control of coleopteran and lepidopteran pests in Europe and North America. During this time microbiologists continued to investigate the precise mode of insecticidal activity for *B.t.*

1.5.2 Diversity of *B.t.* Toxins

The insecticidal components of *B.t.* are proteins which are synthesized during sporulation of the vegetative cell. Several insecticidal proteins may be synthesized together and more may be encoded for in the isolates genome but remain unexpressed. This combination of proteins which comprise the parasporal body (PSB or ICP) is unique to specific subspecies or varieties of *B.t.* and largely determines the target specificity of insecticidal activity (English and Slaten 1992). Therefore, a large amount of the research of *B.t.* has been the identification and classification of *B.t.* isolates and their associated pathogenic spectrum. Classification was originally based upon antigenic composition of the flagella but has been modified to include genetic coding of the PSB and reflect the isolate's insecticidal activity (Hofte and Whiteley 1989). By far the greater number of *B.t.* isolates have shown insecticidal activity to the order Lepidoptera (Fast 1974) however of particular interest to this discussion is the variety *Bacillus thuringiensis* var. *israelensis* (serotype H-14) which has greater toxicity within the order Diptera and is used in Nematocera pest control programs (Becker and Margalit 1993).

Bacillus thuringiensis variety *israelensis* produces the PSB protein inclusion during stage II of sporulation as with other varieties of *B.t.* The PSB is composed of four polypeptide subunits (protoxins) and is spherical in shape (Lerecluse *et al.* 1993). Components of the crystal protein are a 135, 128, and 65 kDa protoxin identified in the literature as *CRY* IV class delta endotoxins and encoded by the *cry* IV class gene (Ibarra and Federici 1986a). Classes of *cry* genes and their associated delta endotoxins are based on entomopathogenic activity and are described by Lerecluse *et al.* (1993). The other protoxin protein which comprise the crystalline structure of

the PSB in *B.t.i.* is a 27 kDa Cyt A protein which has a nonspecific cytolytic activity (Federici *et al.* 1990). Each PSB subunit has demonstrated insecticidal activity individually, but two teams of investigators have demonstrated synergism among the four component protoxins (Ibarra and Federici 1986b, Chilcott and Ellar 1988). Thus, the entire PSB has more insecticidal activity than the sum of its polypeptide subunits.

To date, there are thousands of natural *B.t.* strains which have been isolated and classed into perhaps 30 serotypes (Martin and Travers 1989). Each strain is capable of synthesizing more than one type of crystal which in itself may be composed of several types of protoxin subunits (Lerecluse 1993) creating a wide diversity of bioinsecticides. Recently, hundreds of *B.t.* peptide chains have been described and although there is structural similarity among the many *cry* class of genes and their products, there is a measurable range of toxicity to different insects (Pfannensteil *et al.* 1986). For example, the Cry IV class delta-endotoxins described as A-D had varying levels of mosquitocidal activity among three mosquito species, *Aedes aegypti* Linnaeus, *Anopheles stephensi* Say, and *Culex pipiens* Linnaeus (Bourgouin *et al.* 1986, Thorne *et al.* 1986, Delecluse *et al.* 1991).

1.5.3. Mode of Action

Pathogenesis of *B.t.* toxins occur in several stages upon ingestion of the PSB by susceptible insects. The fact that PSB must first be ingested by insects for any insecticidal effect immediately limits the spectrum of potential target insects since it does not act as a contact poison. The PSB is solubilized into its protoxin subunits in the insect midgut, the process determined by midgut environment (largely pH) and PSB composition (Jacquet *et al.* 1987, Aronson *et al.* 1991). In the case of the 130 kDa subunit protoxins are enzymatically cleaved to produce the actual toxic fragment of 60 - 70 kDa. Choma *et al.* (1990) demonstrated this process *in vitro* using the enzyme tripsin, 10 kDa peptide fragments were cleaved from the carboxyl terminus until the active fragment remained. Further work confirmed insecticidal activity to be the domain of the amino terminus (Chungjatupornchai *et al.* 1988) and the extent that the carboxyl peptides were cleaved co-determined target specificity (Delecluse *et al.* 1991,

Pao-intara *et al.* 1988).

The final determining factor for insect toxicity of *B. t.* delta-endotoxins are the cell membrane receptor sites of the brush border in the insect midgut epithelium (Hofmann *et al.* 1988, Van Rie *et al.* 1990a). These cell membranes have high-affinity binding sites which accept the 20 - 70 kDa proteins and induce the formation of either aspecific or K^+-specific pores (Visser *et al.* 1993) leading to lysis of the cell (Sacchi *et al.* 1986, Hendrickx *et al.* 1990). Specifically, the toxin-receptor complex disrupts osmotic homeostasis at the cellular level allowing ionic imbalance between the lumen and the heamolymph. Feeding paralysis results followed by death of the insect (Yamamoto and Powell 1993).

1.5.4 Ecology and Distribution of *B.t.*

B.t. occurs worldwide, although rarely epizootic among insect populations (Burgess 1973). It commonly persists as spores in soils, insects, dust from material storage facilities and the phyloplane (Martin and Travers 1989, Smith and Couche 1991, Meadows *et al.* 1992). Its ecological role is debated, though it is largely agreed there is selective advantage for insecticidal proteins produced during sporulation (Meadows 1993). At times of unfavourable environmental conditions (*eg.* low nutrient levels, non-optimum pH) spores and associated toxins may remain dormant yet viable for long periods of time. Upon ingestion by insects, if the pH is slightly alkaline and toxins solubilize, bind, and effect gut paralysis, the spore will remain in a nutrient rich environment. Also associated with cytolysis of the midgut epithelial cells is a reduction in pH of the lumen. This effectively enhances conditions for germination of the spore and vegetative growth. Without the crystal toxins, colonization of the insect lumen would be difficult due to rapid transit time and the pH of the insect gut (Ellar 1990). The widespread distribution of *B.t.* is advantageous when considering ecological risk and impact while applying *B.t.* for large scale pest control operations. Since it is enzootic in most environments and entirely free of unnatural chemical residues, it is a very attractive technology in pest management with sensitive ecological applications. At one location in the South Saskatchewan River *B. thuringiensis* colony densities were observed to range between 0 - 1.5 x 10^3/ml within the water column

(Agriculture Development Fund 1990). It is unclear whether the observed density of *B.t.* in the ADF study is at equilibrium and normal to the Saskatchewan River system as an enzootic population or whether this is a remnant population from earlier field trials of *B.t.i.*

1.5.5 Application and Efficacy

There are presently four commercial formulations of *B.t.* subsp. *israelensis* registered and available worldwide (Skeeta, Teknar, Vectobac and Acrobe are produced by Novo Nordisk, Thermo Trilogy, Abbott and Cyanimid respectively). Dozens of other varieties, subspecies and *B.t.* insecticidal products are easily cultured in common media (Dulmage 1983, Devisetty 1993). China and third world countries have demonstrated effective batch cultures at the local level using brans, forages, and blood meal in solid, semi-solid, and liquid fermentation (Kachatourians 1986, Salama & Morris 1993). Parameters inducing sporulation of the bacterium are well understood and concentration of the end product is accomplished by precipitation, centrifugation and ultra filtration (Bernard and Utz 1993). Quality control procedures for the final product are well documented by Groat *et al.* (1990). Therefor, there is a low technology biopesticide as effective as the most active organophosphate insecticides with a much greater specificity for targets.

The present problems associated with *B.t.i.* are in the area of field application technology and the risk of insects developing resistance. Although many problems associated with terrestrial applications do not develop (*ie.* spray droplet size for effective leaf coverage), obstacles unique to aquatic applications required attention. Firstly, *B.t.i.*. precipitates from the water column relatively quickly (with respect to contemporary chemical insecticides) due to its particulate nature. Thus toxins are quickly unavailable for ingestion near the air/water interface where many mosquitos occur. In rivers and streams, toxins are not effectively carried to downstream blackfly populations and necessitate frequent re-applications. Presently these problems are addressed in product formulation by using concentrated liquid formulations in river and stream habitats for more effective transport downstream and slow releasing briquette formulations for mosquitocidal applications.

Secondly, insecticidal activity of *B.t.i.* declines after introduction to water (Becker and Margalit 1993). Identified factors affecting insecticidal activity of *B.t.i.* have been temperature, adsorption to algae and aquatic macrophytes, pH and seston concentration. These factors can affect the physio-chemical properties of *B.t.i.* toxins, the response of target species (*i.e.* ingestion rates) or a combination of the two (Mata *et al.* 1986, Kurtak *et al.* 1989, Costello-Branko and De-Andrade 1992, Coupland 1993, Palmer *et al.* 1996a). Additionally, *B.t.* toxins are known to degrade in response to ultraviolet radiation (Poszgay *et al.* 1987). To compensate for downstream losses of *B.t.i.* Chalifour *et al.* (1990) suggests a calculated 'overdose' of the suggested LC90's. Despite the variables affecting the efficacy, the use of *B.t.i.* in blackfly control programs has been largely successful (50-85% mortality in the aforementioned trials). Efficacy trials for immature blackflies are still being reported for a wide range of lotic conditions and commercial formulations, mortality rates vary but can approach 100% (Lacey and Heitzman 1985, Welton and Ladle 1993, Abergast and Rupp 1994, Palmer *et al.* 1996a).

1.5.6 Impact of *B.t.i.* to Nontarget Organisms

Bacillus thuringiensis is an insecticide specific to herbivorous insects and, in the aquatic environment, filter feeders. It is biodegradable and safe for virtually all vertebrates and plants (Burgess 1993). The degree of impact to non-target organisms observed in aquatic field applications of *B.t.i.* varies somewhat among reports, depending on the scope of the study and the importance placed on components of the aquatic community. In cases where trials were largely designed to record blackfly mortality and associated drift of nontarget organisms, results are reported as minimal or negligible (Yameogo *et al.* 1988, Welton and Ladle 1994). Experiments of perhaps more sensitive design have reported mortality of some species of Chironomidae and Tipulidae (Diptera) and Heptageniidae (Ephemeroptera) (Jackson *et al.* 1994, McCracken and Mathews 1997, Pistrang and Burger 1984, Wipfli and Merrit 1994a). Mortality of some filter feeding Diptera can be expected due to susceptibility of all four toxins present in *B.t.i.*. In the case of the ephemeropteran mortality, experimental dosages were significantly higher than those recommended. Wipfli and Merrit (1994a, 1994b) have done the most comprehensive studies to date involving 16 taxa in artificial streams and field applications

of *B.t.i.*. They observed most species did not respond directly or indirectly to *B.t.i.* and that predators in general do not suffer toxicosis from *B.t.i.* killed animals. Specialist predators of simuliid larvae ie., *Acroneuria lycorias* (Perlidae) were affected by the absence of prey, however generalist predators will switch to alternative prey species and remain unaffected.

1.5.7 Insect Resistance to *B.t.*

The development of resistance to previously effective insecticides is always a concern to pest managers and *Bacillus thuringiensis* is no exception. Resistance to *B.t.* has been observed in laboratory and field populations of ten species (Selinger 1993). Van Rie *et al.* (1990b) has determined the mechanism of resistance in the case of *Plutella xylostella* Linnaeus to be due to changes in specific receptors in the cell membranes of the insect midgut (ICP receptors). These observations were made with laboratory and field populations of *P. xylostella* and similar findings supported this general mechanism of resistance in field populations of *Plodia interpunctella* Hübner (Van Rie 1993). Changes in ICP receptor sites were not correlated with resistant strains of *Heliothis viriscens* Fabricius suggesting another mechanism may be involved (MacIntosh *et al.* 1991). In the case of *H. viriscens*, cross resistance to several delta endotoxins occurred in the resistant strain (Gould *et al.* 1992).

The presence of more than one *B.t.* toxin appears to be a major factor in the ability of insects to develop resistance (Mani 1985), although this statement may be too general for all pests. In the aforementioned cases, *B.t.* products with only one or two toxins present (CryI and CryIIA) were used on isolated populations in laboratories and grain storage bins. In cases of Nematocera control with the four toxins present in subspecies *israelensis*, incidence of resistance are far less frequent. Georghiou *et al.* (1993) has demonstrated *C. quinquefasciatus* develops only low-level resistance (1.6 fold) to *B.t.i.*. with all four toxins present (28, 65, 125 and 135 kDa). Using a mixture of only three toxins (65, 125 and 135 kDa) resulted in significantly higher levels of resistance (7 fold). In long term, large scale field programs such as control of *Aedes vexans* in the Upper Rhine valley (Becker and Ludwig 1993), and the onchocerciasis program controlling *Simulium damnosum* in West Africa (Guillet *et al.* 1990), no resistance has yet been observed.

2. DESCRIPTION OF THE STUDY AREA

2.1 Saskatchewan Blackfly Control Zone

The study area where Saskatchewan blackfly control efforts have recently focussed is a corridor of approximately 20,000 km^2 surrounding the Saskatchewan River system (Figure 1). This area has been identified due to mass emergences of blackfly pest populations, primarily *S. luggeri*, as well as an area of livestock production which has historically sustained the brunt of the economic impact (Fredeen 1985a). The area encompasses the municipalities of Duck Lake and Carlton near the South Saskatchewan River, east to the municipalities of Melfort and Tisdale. The northern border of the zone is delineated primarily by the Northern Provincial Forest with communities of concern being Shellbrook to the west and townships along Highway 55 east to Nipawin (*eg.* Meath Park, Smeaton and Choiceland). The area also contains numerous smaller watersheds and streams (*eg.* Whitefox River, Torch River, Bisset Creek and Bedard Creek, Carrot River) which contribute to the adult blackfly population and can generate localized outbreaks (Mason and Kusters 1990a). However, from sampling adult pest populations in the field and correlating observations with larval population trends observed in surrounding watersheds, the Saskatchewan River system has been identified as the primary larval habitat generating the most significant proportion of biting blackflies (Fredeen 1985b, Mason and Kusters 1990b). Therefore, blackfly control efforts are focussed on larval population monitoring and larvicide applications to approximately two hundred kilometres of the Saskatchewan River system. In addition, approximately sixty kilometres of the Torch River is routinely monitored and treated with blackfly larvicide if deemed necessary by the program coordinator. Economic thresholds described by Fredeen (cited in Mason and Kusters 1990b) of 1000 larvae/meter of artificial substrate provide the basis for determining larvicide applications to the Saskatchewan and Torch Rivers. Larvicide applications are regulated by Saskatchewan Environment and Resource Management and require an annual permit for the control of aquatic nuisances.

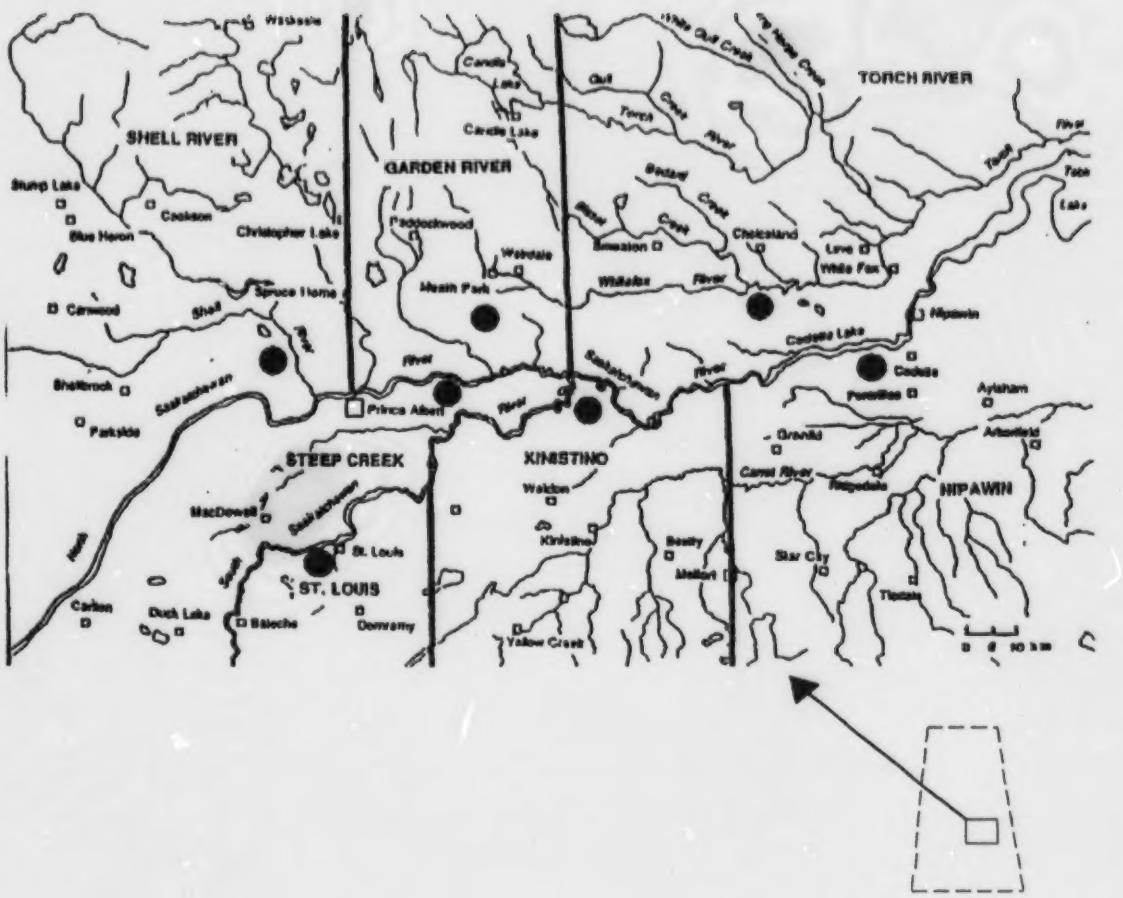


Figure 1. Area of East Central Saskatchewan delineated as the blackfly control zone because of agro-economic impact from blackflies (Diptera: Simuliidae). Dots represent 7 sampling locations for adult blackfly collections. Lower illustration = Saskatchewan.

2.2 The Saskatchewan River System

2.2.1 The North Saskatchewan River.

The North Saskatchewan river originates from meltwater and tributaries draining the eastern watershed of the Columbia Icefields. In Alberta, the confluence of the Clearwater River, the Brazeau and the North Saskatchewan drain a total area of 152,030 km^2 (Jarvis 1987) before joining the South Saskatchewan approximately 40 km east of Prince Albert, Saskatchewan. Through Saskatchewan the river profile is characterized by turbid water flowing slowly through glacially deposited soils, sand and till. This characterization is modified by annual run off cycles which dramatically increase river discharge in April, primarily from prairie meltwater, and again in late June from mountain meltwater. Within the study zone, downstream of Prince Albert, the river is depositional with predominantly sandy substrate although numerous gravel bars are present. It is relatively shallow, approximately 1 to 3.5 m deep, and between 200 and 500 meters wide. Approximately 35km downstream of Prince Albert there is a transition to erosional habitat where the river substrate is larger cobble and rock.

The river is impacted from the municipalities of North Battleford and Prince Albert which currently utilize only primary sewage treatment technology, and the Weyerhauser Pulp and Paper facility which discharges effluent into the river approximately10km downstream of Prince Albert. These impacts are summarized as allochthonous nutrient enrichment which has yet to be quantified (Golder Associates 1996).

Larvicide applications occur over approximately sixty kilometers of North Saskatchewan River larval habitat downstream of Prince Albert. This involves routine monitoring of larval populations by artificial substrates from six established stations illustrated in Figure 2. Blackfly population sampling relies on artificial substrates suspended in the water column as a means to standardize samples. *S. luggeri* typically reside upon aquatic macrophytes and suspended snags but the collection of natural substrates make density measurements difficult to standardize.

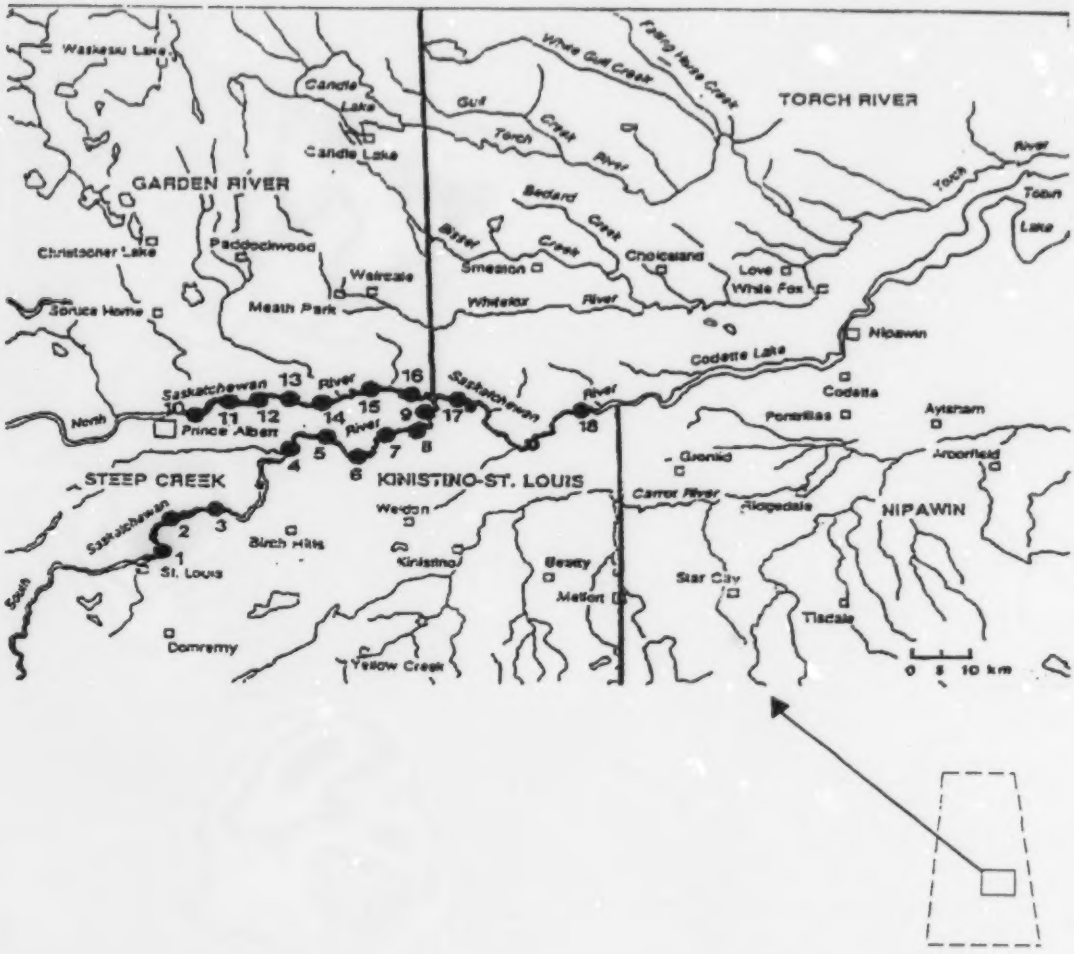


Figure 2. Sampling locations for the routine collection of blackfly larvae from the Saskatchewan River 1988-2001. Locations visited on a weekly basis during ice free conditions for larval population monitoring with the aid of artificial substrates. Lower illustration = Saskatchewan.

Placement of artificial substrates (monitors) and collections begin immediately following ice break up in late April or early May and artificial substrates are collected on a weekly basis until September. Eggs of *S. luggeri* hatch shortly after ice break up (Jarvis 1987, pers. obs.) and first instar larvae were observed upon substrates in early to mid May. The preliminary spring population of *S. luggeri* is loosely synchronized in development compared with future cohorts and generations. When the density of larvae exceeds the economic threshold established by Fredeen (cited in Mason and Kusters 1990b), with the majority of individuals in the 4th instar with few or no individuals reaching the 7th instar, *B.t.i.* larvicide applications commence.

The efficacy of each *B.t.i.* application on the North Saskatchewan River was closely examined for the 33 km of North Saskatchewan River immediately downstream of the City of Prince Albert. In addition to the routine larval population monitoring, 13 artificial substrate monitors are placed equidistantly through the treatment zone in mainstream discharge (Figure 3). This supplemental data is a more comprehensive analysis of larval mortality and provides a critical evaluation of how effectively the area was treated.

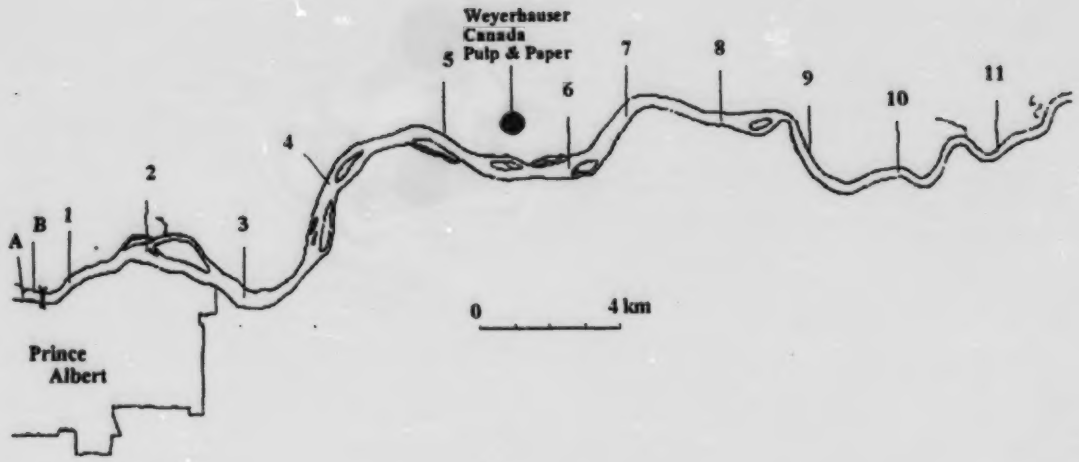


Figure 3. The North Saskatchewan River study area illustrating 13 sampling locations for the collection of artificial substrates before and after application of blackfly larvicide *Bacillus thuringiensis* variety *israelensis*. Locations A and B served as upstream reference stations with 11 downstream stations within the impacted area.

2.2.2 The South Saskatchewan River

The South Saskatchewan River enters Saskatchewan from the confluence of the Bow, the Old Man, and the Red Deer Rivers in Southern Alberta.. There is a major impoundment of the river by a hydroelectric facility near Macrorie Saskatchewan forming a large reservoir known as Lake Diefenbaker. Jarvis (1987) notes this facility has a dramatic effect on the South Saskatchewan River and associated fauna as water is held back during spring run off so as to be released in times of peak power consumption in winter. This essentially reverses the natural hydrological cycle downstream of the facility. In addition to reversal of discharge rates, the reservoir serves a sink for the seston load of the system. The river is now quite shallow and clear in the spring and summer months allowing the development of extensive weed beds. The four most common macrophytes being *Ceratophyllum demersum* Linnaeus, *Myriophyllum exalbescens* Fernald, *Potamogeton crispus* Linnaeus and *Potamogeton pectinatus* Linnaeus and the algae *Cladophora glomerata* Kutzing (Fredeen 1985a). These changes in the river profile have been implicated in the colonization of the Saskatchewan river by the economically important *S. luggeri* (Fredeen 1977b) as well as the difficulties in effectively treating it with the larvicide *B.t.i.* (Lehmkuhl 1990). The Saskatchewan blackfly control program monitors blackfly larval populations using artificial substrates collected from 9 established stations over 100 km of the river (illustrated in Figure 2). Monitoring begins immediately following ice break up in April or May and continues on a weekly basis until September.

3. METHODS AND MATERIALS

3.1 Larval Population Sampling

The Saskatchewan Blackfly Program routinely collects artificial substrate samples and /or natural vegetation for blackfly larvae on a weekly basis from ice breakup in April or May until September. A total of 24 locations were established and routinely sampled on a weekly basis to evaluate species composition and age class of blackfly larval populations of the Saskatchewan and Torch Rivers. The data collected provide an estimate of the temporal and spatial distribution of the blackfly larval population and is the basis for determining larvicide applications (Mason and Kusters 1990b). Observations allow timely larvicide applications before pupation or adult emergence as well as documenting the recovery of blackfly larval abundance after *B.t.i.* treatments.

Supplemental to the routine weekly sampling from 24 locations, 13 additional sampling locations were utilized on the North Saskatchewan River for *B.t.i.* efficacy data. These samples were collected before and after each *B.t.i.* application on the North Saskatchewan River downstream of Prince Albert and were essential in evaluating the effect of *B.t.i.* concentration (dosage) on the larval population. Supplemental efficacy observations provide a more critical evaluation of the immediate effect of *B.t.i.* applications upon blackfly larval densities whereas weekly sampling estimate the seasonal trends in population densities. The 13 supplemental sampling locations provided 39 observations of change in blackfly densities from *B.t.i.* exposure and reference locations. By varying the dosage of *B.t.i.* in this treatment zone the investigation described mortality rates (change in blackfly densities) at given *B.t.i.* dosages more precisely and thereby optimize the cost effectiveness of the program's larvicide operations. This is imperative for the program's success within a restricted operating budget given the increased cost of biological larvicide. Had differences in observed blackfly mortality not been explained by the *B.t.i.* concentration variable, control or non-exposed abundance measures would be relied upon to explain observations.

Every effort was made to collect the complete series of data each trial regardless of weather and river conditions. At times, artificial substrates and monitors were lost or destroyed due to extreme discharge rates, debris entanglement or vandalism.

3.1.1 Population Monitoring Using Artificial Substrates

The methods used for sampling the blackfly larval population were developed for the Saskatchewan River system by Fredeen and Spur (1981). Standard lengths of 3/8 inch polypropylene ropes were used as artificial substrates to collect drifting blackfly larvae in Fredeen and Spur's (1981) study. The sampling is a semi-quantitative estimate of the drifting simulid population and substrates are comparable replicates but lack definitive quantification with respect to the natural standing crop of the habitat being sampled. Each rope replicate was 1m in length and attached to a float which is anchored in place at monitoring locations. The artificial substrates were attached by a 'snap' swivel and allowed to trail freely where they remained submerged just under the water surface to simulate aquatic vegetation. Jarvis (1987) modified this sampling method with the use of 1m lengths of Weld Loc TM polyethylene reinforcement tape which provided a more consistent surface area than the braided rope used by Fredeen (1978). This project continued to use reinforcement tape during this sampling regime since blackfly larval densities were significantly higher on smoother surfaces (Fredeen and Spur 1981). Higher densities of larvae that colonized the artificial substrate reduced sample variance and thereby increased sampling efficiency (Morin 1985).

For the 24 sampling locations referred to as 'routine' monitoring of the blackfly larval population, each monitor consisted of three polyethylene substrates (Figure 4). Substrate assemblies were placed in a range of river depths and current velocities based on accessability of the location. Typically, South Saskatchewan River locations are accessed by a vehicle and a collector wearing chest waders. North Saskatchewan River stations are typically in mainstream discharge, hence maximum depth and current velocity, and collections are made with a water craft. On a weekly basis, each monitor is visited and a substrate is removed and replaced with a new substrate. Substrate Number 1 is collected and replaced the first week, followed by Number

2 the second week and so on. The rotation returns investigators to Tape #1 on the fourth week and allows each tape to remain in the water column for three weeks for colonization of blackfly larvae. However, no statistical assumptions were violated, and the Log 10 of all weekly observations were taken as a means of standardization.

The three week conditioning, or colonization time, is a compromise between Saskatchewan River conditions and allowing artificial substrates to most accurately reflect the standing crop of blackflies within the habitat. Minshall and Peterson (1985 cited in Jarvis 1987) examined benthic invertebrate colonization of 'habitat islands' in European temperate streams. The model which they describe suggests microhabitats such as artificial substrates should develop a stable, representative community in approximately 4 weeks. Artificial substrates which remain in the Saskatchewan River beyond two or three weeks become coated with algae, detritus and sediments and become less desirable as substrate for blackfly larvae attachment (pers. obs.). Therefore a 3 week colonization period seemed an appropriate balance between these factors. Upon collection, artificial substrates were placed in a Mason jar containing 70% ETOH. Samples were labeled with the date, location, tape number and collectors initials and returned to the laboratory for enumeration and identification of preimaginal blackflies.

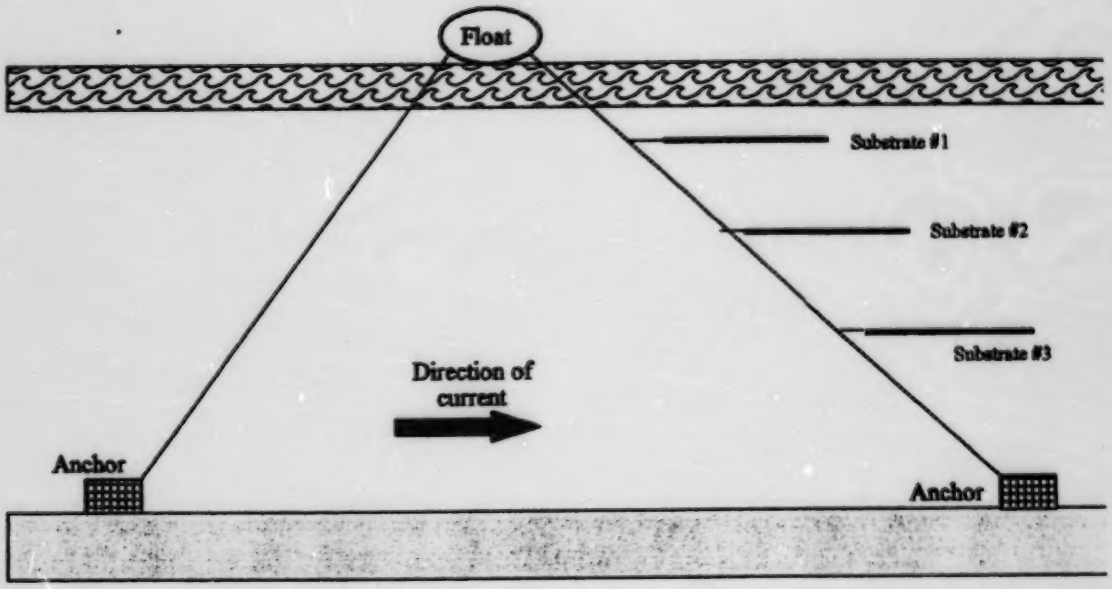


Figure 4. Diagram of artificial substrate assembly used for the collection of blackfly larvae in this study for routine population estimates. 1 substrate was collected and replaced each week on a rotational basis. Thereby substrates were allowed to colonize for a three week period prior to collection.

3.1.2 Efficacy Sampling

Artificial substrates used to determine the efficacy of *B.t.i.* treatments in the North Saskatchewan River were deployed in the river after routine monitoring of the area confirmed that *S. luggeri* larvae had hatched, developed, and reached densities where *B.t.i.* applications would commence the following week. The densities used as thresholds for larvicide applications were 1000 larvae/meter of substrate and intuitively established by Fredeen (1983) and described in Mason and Kusters (1990b). This allowed five days for colonization of the substrates in each efficacy trial. Adequate numbers of simuliid larvae colonized substrates to provide measures of mortality but the relatively short conditioning time would not allow the artificial substrate to accurately represent the standing crop of the simuliid assemblage (Minshall and Peterson 1985 cited in Jarvis 1987). This is of little importance to overall examination of the effect of *B.t.i.* since these mortality measures are complimented by routine population monitoring utilizing a 3 week colonization period. The efficacy monitors used were consistent in design with those previously described (Section 3.1.1) except in the number of attached substrates. Each of the 12 monitors were constructed with a 6m, 1/4 inch anchor rope to the float, and a 10m, 1/4 inch attachment rope for 6 - 1m x 1cm lengths of Weld-Loc TM polyethylene reinforcement tape (Figure 5). Artificial substrates were attached using twisted copper wire (12 ga. 'Snare' wire) threaded through the braid of 1/4 inch polypropylene rope. All efficacy substrate assemblies were placed in mainstream river discharge between depths of 2 - 3m.

Pre-treatment substrates were collected 2 - 4 hours prior to the *B.t.i.* application to the area, which is the time required for the collection of all substrates distributed over the study area. Substrates were collected from a boat using wire cutters below the water surface to minimize the disturbance to other substrates. Three pre-treatment tapes were collected from each station from 3 levels within the water column (surface, mid depth and bottom). Even numbered tapes were collected from the first station followed by odd numbers the next station, alternating downstream. This was to avoid sampling each surface tape with the pre-treatment collection or every bottom substrate among the post-treatment collection. Measurement of blackfly

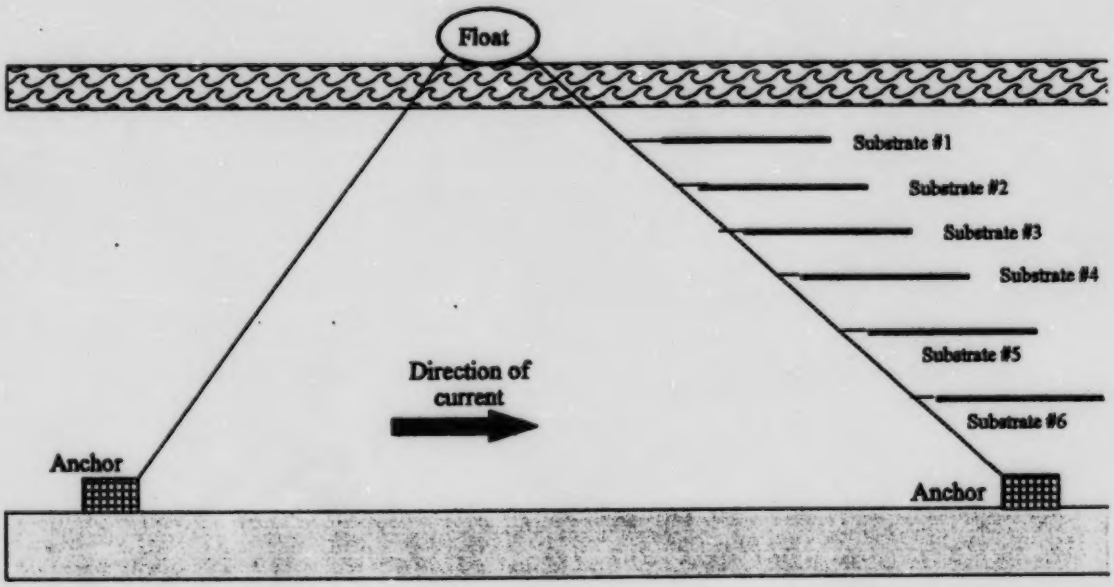


Figure 5. Diagram of artificial substrate assembly used for the collection of blackfly larvae in this study for the efficacy data. Odd numbered substrates were collected prior to *B.t.i.* application and even numbers were collected 48 hours following treatment, alternating the procedure at the next location. Thirteen assemblies at exposure and reference locations were utilized for 4 *B.t.i.* dosage rates.

'mortality' from efficacy substrates were derived from pre and post treatment comparisons which were made from nearest depth observations (*ie.* substrate #1 was compared with #2, #5 with #6 *etc.*). Hence 3 measures of mortality were determined for 3 river depths, averaged, and a non-parametric (distribution free) analysis was used to examine the data.

3.1.3 Blackfly Drift Estimate

At the time of pre-treatment collection, another clean polyethylene tape was attached near the water surface for a relative measure of drifting larvae during the *B.t.i.* treatment within the study area. Since *B.t.i.* is only effective when ingested by sessile larvae, a more accurate assessment of mortality can be calculated by including an estimate of that portion of the larvae which are at drift during time of *B.t.i.* application (Undeen and Lacey 1982). The new substrate at each location provided an estimate of blackflies recolonizing substrates during the 48hr period of *B.t.i.* trial and post treatment collection. It should be indicative the portion of the blackfly population at drift during the application. The post-treatment substrates and blackfly drift estimate, were collected 48 hours after the *B.t.i.* application. Samples were appropriately labeled and preserved with 70% ETOH for laboratory enumeration and identification.

3.2 Laboratory Procedures

3.2.1 Sorting

Field samples were enumerated and identified in the laboratory under 12X magnification using a Wild stereoscopic microscope. All blackfly larvae and pupae were removed from substrates and placed into a petri dish containing 70% ETOH. The remaining sample was sieved (502 μm) and also placed in the petri dish. Gentle agitation of the dish was used to sort animals from the sample debris. The sample debris was then re-agitated and remaining blackflies were removed. If blackflies were present in the subsequent agitation, the process was repeated until no animals were discovered. This method has been reported to successfully remove 95 - 99% of all

organisms within a sample (TAEM 1997). 10 samples (14%) of the pre and post efficacy samples were resorted in the preliminary trial to confirm sorting efficiency (APPENDIX 2). No additional blackflies were found within fines and coarse debris.

3.2.2 Subsampling

Subsampling was avoided in most cases for the efficacy trials, however, during routine monitoring of the Saskatchewan River some unmanageably large samples were collected. In these instances samples were sorted in the same manner described in Section 3.2.1 to remove debris, then subsampled using an Imhoff cone technique (Wrona *et al.* 1982). The apparatus consists of an Imhoff sewage settling cone containing ethanol and equipped with an air stone at the bottom. An aquarium type air pump is connected to the air stone by rubber tubing to keep ethanol and blackflies mixing uniformly . A twenty milliliter test tube was used to withdraw 2 subsamples and placed in separate petri dishes. Each subsample was enumerated and an average of the 2 subsamples was calculated to provide an estimate of species composition, larval instar distribution and relative abundance. The average estimate was then multiplied by the inverse of the fraction taken from the original volume. A minimum of 20% of the whole sample was counted in each subsample procedure to ensure repeatability from sample to sample as well as maintain sample efficiency. Where such sub-sampling was used, the fraction to which the sample was split was recorded.

3.2.3 Determination of Species and Larval Instars

All blackfly larvae were identified with respect to taxonomy and instar maturity due to the importance of targeting only economically important species in a correct stage of development for larvicide applications. Taxonomic identification of immature blackflies was accomplished using F.J.H. Fredeen's keys for the Saskatchewan River (Fredeen 1981) and a stereoscopic microscope with 25X magnification.. The keys describe phenotypes of late instar larvae and in the case of very early instar larvae, higher magnification and the experience of the author was

relied upon. In these cases, *S. luggeri*, *S. meridionale*, and *S. vittatum* are easily distinguished and only with early instar *S. arcticum* complex, *S. tuberosum*, and *S. venustum verecundum* complex could some confusion exist. These species are relatively rare in the North Saskatchewan River with respect to other species and if early instar larvae could not be positively identified, they were lumped with *S. luggeri*.

The seven larval instars of *S. arcticum*, *S. luggeri*, and *S. vittatum* have been described with their associated post genal lengths (Fredeen 1976, 1981 and Ross 1979 respectively). As well, the mean whole body length for each instar of *S. meridionale*, *S. tuberosum*, and *S. venustum / verecundum* complex have been described by Mason and Kusters (1990b). Thus, instar development was determined for each blackfly larvae with a micrometer on the eyepiece of the microscope at 25X magnification.

3.3 *B.t.i.* Application to the Saskatchewan River

3.3.1 Calculation of the *B.t.i.* Dose

For large rivers with high seston loads, it has been suggested that a dose of 25 parts per million *B.t.i.* be applied for 1 minute at a point injection within the river (Colbo 1984). However, Mason and Kusters (1990b) suggest point injections of *B.t.i.* at lower concentrations which are sustained for longer periods of time to be more effective over 10 km of downstream habitat in the Saskatchewan River. The calculation for the volume of *B.t.i.* required relies on knowing the precise discharge of the river to be treated. For the North Saskatchewan River, this information can be obtained from the automatic staff gauge in Prince Albert operated by Environment Canada. The reading of the staff gauge is then correlated to discharge with Stage - Discharge Tables provided annually from Environment Canada. Once the discharge is known and application rate (dosage) has been decided, the following calculation determines the volume of larvicide needed:

$$\text{Volume}_{B.t.i.} = \text{Discharge} \times \text{Application Rate}$$

For example: on May 31, 2000 the North Saskatchewan River discharge was 266 m^3/ s, the volume of *B.t.i.* required for a dosage of 1.0 ppm sustained for a 15 minute period would be:

$$\text{Volume}_{B.t.i.}\ (\text{liters}) = 1.0/10^6 * 900\ s * 266\ m^3/s * 1000\ l/m^3$$

$$\text{Volume}_{B.t.i.} = 240\ \text{liters}$$

3.3.2 Delivery of *B.t.i.*

Conventional methods of larvicide applications to river habitat utilizes a vehicle (*e.g.* boat or helicopter) to release a predetermined dose upstream of blackfly pest populations (Colbo 1984, Fredeen 1974, 1975, Gibbs *et al.* 1986). This is referred to as a point injection and relies on the river current to deliver the active ingredient to downstream targets. Applying *B.t.i.* with this technique normally involves overdosing at the point of application to compensate for downstream losses of active ingredient (Chalifour *et al.* 1990; Merrit *et al.* 1989) Numerous factors inhibit the delivery of a lethal dose of *B.t.i.* by this method, primarily *B.t.i.* particles falling from the water column due to competition with other suspended particles or low current velocity. Deterioration of toxins from UV light and adsorption to plants and debris within the river may also be factors (summarized in Section 1.6.5). Lehmkuhl (1990) observed greater success delivering lethal doses of *B.t.i.* to problem areas of the South Saskatchewan River by undertaking a pro-active delivery of the larvicide. This involved navigating a boat over the target habitat while releasing the larvicide dose. The release of the dose was regulated allowing more product to be released upstream of rapids or critical habitat and less released in calm areas or back eddies.

In the North Saskatchewan efficacy trials, this latter method was adopted with the intention of more uniform coverage of the treatment zone. Although the study area is relatively a uniform depositional habitat, this application method provides better dispersal over dead zones and back eddies. Dosages were calculated as described in Section 3.2.1 using 4 concentrations of Teknar HPD ® *B.t.i.*, 2.5 ppm (2001), 2.0 ppm, 1.5 ppm, and 1.0 ppm (2000) for a10km section of river (Mason and Kusters 1990b). Thus four doses were applied to the North Saskatchewan River study area, with the fourth dose beginning at kilometer 30 and being released over 6 km beyond the final efficacy substrates. The boat was navigated in a zig-zag

pattern downstream at approximately uniform speed and uniform release of the *B.t.i.* throughout the treatment area. Equipment used was a 20 foot flat bottom boat with a 85 hp jet drive outboard motor, a 1000 litre polyethylene water tank for holding larvicide dosages, a 3.5 hp Honda pump to release the larvicide, an Insight flow meter for observing output rates, and an assortment of plastic hoses, stopcocks and fittings.

3.4 Data Management and Analysis.

3.4.1 Efficacy Data

The efficacy data presented here is a standardized index of mortality (Δ Density) from 7 relative measures of abundance using artificial substrates at 12 locations. 3 pre treatment measures were matched, with respect to depth in the water column (*ie.* surface, mid and bottom samples) and compared to 3 post treatment measures. This comparison was modified with a corrective measure of the drifting population (described in section 3.1.3) in the form:

$$\Delta \text{ Density (\%)} = \frac{\text{(Pre treatment density + drift) - post treatment density}}{\text{(Pre treatment density + drift)}}$$

Thus, 3 estimates of mortality (Δ Density) from 3 depths were observed for 12 monitoring stations using artificial substrates. Examination of the frequency distribution of density changes from sampling trials illustrated cases of heteroskedasticity and bimodality of the data. In addition to the evidence of a non parametric distribution of the efficacy data, Green (1979) suggests most one tailed parametric tests are far less robust to violations to their assumptions. Therefore a distribution free or non parametric analysis was more suitable for the efficacy data. Since observations were derived from matched replicates, and we hypothesized *a priori* an ordering of mortality measures with respect to treatment dosage, a Page Test of Ordered Alternatives was appropriate (Siegal and Castellan 1988).

The null hypothesis to be tested (H_o) states: "The median change in density (θ) of blackfly larvae calculated from artificial substrate sampling is the same for each larvicide treatment, that the

relative mortality observed is independent of *Bacillus thuringiensis* variety *israelensis* concentration in the North Saskatchewan River discharge".

$$H_o: \ \theta_1 = \theta_2 = ... = \theta_k$$

The alternative hypothesis (H_1) therefore is that the median change in density of blackfly larvae calculated from artificial substrate sampling is not the same for each treatment and the relative density change measured is dependant upon *B.t.i.* concentration, specifically, treatments with greater concentrations of *B.t.i.* in the North Saskatchewan River discharge result in greater reduction of blackfly densities.

$$H_1: \ \theta_1 \neq \theta_2 \neq ... \neq \theta_k$$

Let the level of alpha (α) for the rejection of H_o be 0.05, N = 33, and the number of treatments (k) = 4. We assumed the reduction of blackfly larval density to be greater with higher concentrations of *B.t.i.* larvicide, the test of ordered alternatives is therefore a one-tailed test. In the case of different median changes in density being detected, and H_o is rejected, multiple comparisons among treatments will be conducted to determine which treatments differed significantly with $\alpha = 0.05$ (Siegal and Castellan 1988). In the case of no differences detected between treatments, and H_o cannot be rejected, comparisons between treatment observations and reference (control) observations would be interpreted.

3.4.2 Routine Larval Population Data

The raw data collected from routine collections of artificial substrates was transformed logarithmically prior to summary and presentation. This was done because of the spatial distribution of samples (> 40km of North Saskatchewan River) and would prevent an unusually high abundance of blackfly larvae from one location biasing the sample mean (Sokal and Rohlf 1995). Green (1979) suggests most biological data require some type of data transformation albeit in the context of natural populations rarely meeting the assumptions of parametric statistical tests. Jarvis (1987) employed a logarithmic transformation using the base 10 to ease interpretation of the his artificial substrate data and we have conformed to this presentation.

The observations used to calculate mean larval abundance and the associated standard error was therefore:

$$Y' = \mathrm{Log}_{10}(Y + 1)$$

The mean larval abundance from 5 replicate artificial substrates collected weekly from the North Saskatchewan River from ice break up until August 15 of each year and their associated standard errors are presented. All immature blackfly population observed from artificial substrates are presented in the data and hence pupae are included. Pupae are not feeding during this development phase and are therefore unaffected by *B.t.i.*. Interpretation of the routine monitoring data should be made keeping in mind greater abundance of pupae later in the season. The data were plotted in two dimensions, abundance versus time, for illustration of the expression.

4. RESULTS

4.1 Effect of *B.t.i.*

The Saskatchewan river became ice-free during the third week of April in 2000 and 2001. Artificial substrates were deployed throughout the river at 24 routine monitoring locations in the last week of April with the first samples being collected in the first week of May. *S. luggeri* were abundant in samples collected May 11th and 15th sufficient to warrant *B.t.i.* application to the efficacy study area. Three efficacy trials were completed in the year 2000 at dosages of 1.5ppm, 1.0ppm, and 2.0ppm. The 4th trial at 2.5ppm was conducted on June 29, 2001. The environmental variables and dosages of these trials are summarized in Table 1.

4.1.1 1.5 ppm

Efficacy substrates were deployed on May 12 with the preliminary trial of 1.5ppm *B.t.i.* scheduled for May 18th. On May 18 the North Saskatchewan River discharge was 180 m^3 /s and the calculated dosage for 1.5 ppm *B.t.i.* sustained for 15 minutes required 243 liters for 10 km of river. This was rounded up to 245 L per which required 1220L of *B.t.i.* to be applied over the entire study reach. Pre-treatment substrates were collected immediately prior to treatment with the post treatment and drift estimates collected 48 hours later on May 20th. All samples (N = 91) were recovered in this trial and returned to the laboratory for enumeration.

The mean mortality calculated for 1.5ppm *B.t.i.* was 0.699 or 70% for the study area with a standard deviation of 0.28 (n = 33). The estimate of drifting larvae for this trial was relatively low, ranging from densities of 319 at reference Site A to 49 at Station 9 in the exposure area. There was a distinct trend of lesser recolonization or drifting larvae downstream. This trend in conjunction with an apparent cumulative effect of *B.t.i.* downstream resulted in higher mortality measures at downstream locations compared with upstream exposure areas. The longitudinal (downstream) effect of *B.t.i.* is illustrated in Figure 6.

[*B.t.i.*]	1.0ppm	1.5ppm	2.0ppm	2.5ppm
***B.t.i.* Application**	May 31	May13	June 18	June 29
Discharge (m^3/s)	266	180	455	275
Temperature	15 °C	12 °C	16 °C	15 °C
***B.t.i.* Dose per 10km (liters)**	240	243	819	682
Total *B.t.i.* Applied (liters)	1200	1220	4090	3410
Mean Mortality	63.8%	70.0%	89.5	84.3
Standard Deviation	30.6%	27.9%	12.3%	19.1%
Sample Size (N)	33	33	33	33

Table 1. Summary of 4 blackfly efficacy trials using biological larvicide *Bacillus thuringiensis* variety *israelensis* on the North Saskatchewan River downstream of Prince Albert, 2000 - 2001. Dosages determined from calculating desired concentration (parts per million) within river discharge (m^3/s) for 15 minutes. Relative mortality of blackfly larvae determined from change in blackfly densities of polyethylene artificial substrates collected before and after *B.t.i.* application.

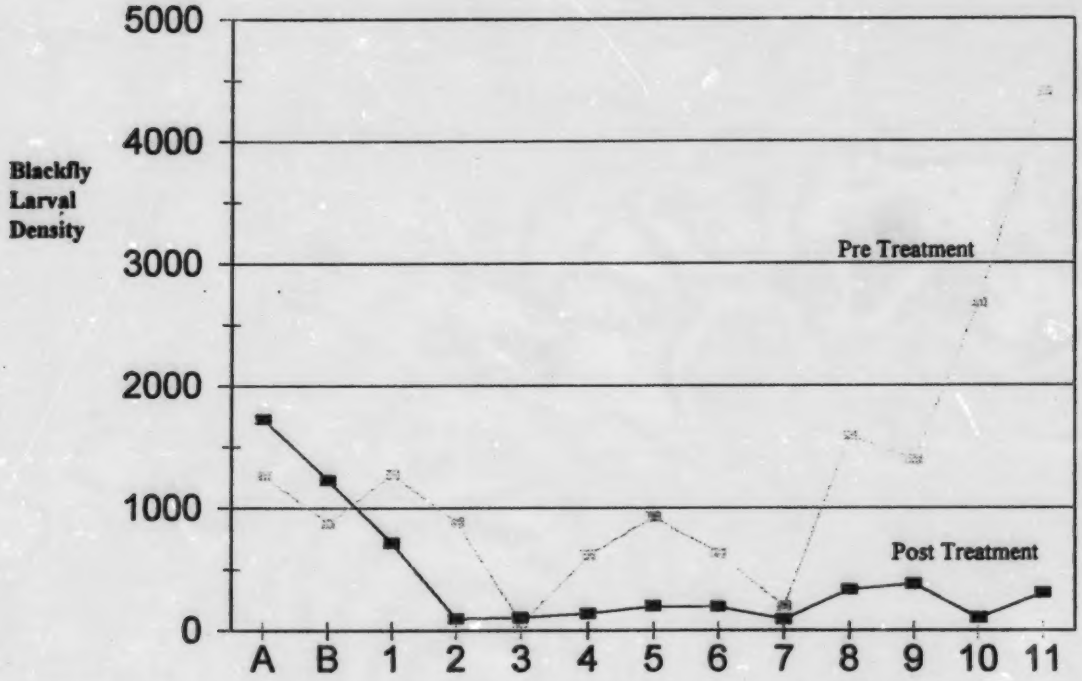


Figure 6. Mean blackfly larval densities observed at 11 exposure stations located equidistantly along 33km study reach of North Saskatchewan River, and; A and B - unexposed reference location upstream of treatment area. Observations collected from artificial substrates before and after treatment with 1.5ppm *B.t.i.* May 18, 2000. Means were calculated from 3 mortality measures from substrates at 3 depths (n=3, N=39).

4.1.2 1.0ppm

Blackfly larval densities increased over the following 2 weeks after the preliminary *B.t.i.* trial until greater than the economic thresholds described for the region. Efficacy substrates were placed at the same 13 locations as the preliminary trial including the 2 reference stations. The 1.0ppm trial took place on May 31st with the North Saskatchewan River discharge at 266m^3/sec. Thus, 5 doses of 240 liters were to be applied to the study area or 1200 liters in total. All pre-treatment, post treatment and drift estimates were successfully collected in the 1.0ppm trial.

The mean mortality observed with 1.0ppm *B.t.i.* over the study reach was 0.638 or 64% and an associated standard deviation of 0.31. This was the highest variance observed for all trials and although drift density measures were also variable, the observations suggest inconsistent results with this dosage. Observed measures of mortality ranged from zero in several locations and depths, to 98% at Station #9 (5-6m depth). A high degree of variability was observed in pre treatment larval densities which certainly contributed to the range of mortality observations. The trial was consistent with preliminary results at 1.5ppm in that there was a trend for higher mortality at downstream locations which may support a cumulative longitudinal effect (Figure 7).

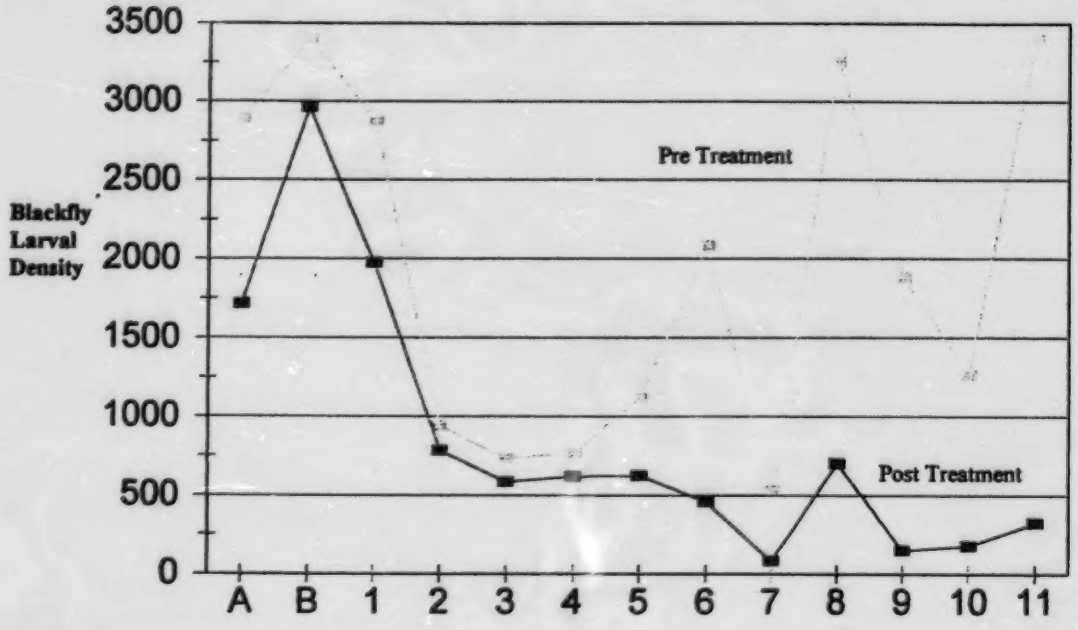


Figure 7. Mean blackfly larval densities observed at 11 exposure stations located equidistantly along 33km study reach of North Saskatchewan River, and; A + B - unexposed reference location upstream of treatment area. Observations collected from artificial substrates before and after treatment with 1.0ppm *B.t.i.*, May 31, 2000. Means were calculated from 3 mortality measures from substrates at 3 depths (n=3, N=39).

4.1.3 2.0ppm

During the month of June the North Saskatchewan River discharge increased steadily with meltwater from the Columbia Icefields and above average precipitation on the prairies. The 2.0 ppm trial was conducted on June 21 with the river discharge at Prince Albert measuring 455m^3/sec. Dosage volumes required for this concentration were 819L per 10km reach for a total applied volume of 4090L. Each of the 91 artificial substrates were deployed and recovered in the 2.0ppm trial completing the full range of observations for the trials in 2000.

The mean measure of mortality observed at 2.0ppm *B.t.i.* was 0.895 or 90% with a standard deviation of 0.12. Although river discharges were larger than previous trials, the larval population appeared relatively stable with lower than average measures of drift densities. The mortality measures were the most consistent of all trials to date. The first exposure station (#1) had mortality measures ranging from 50 - 58% but all others were above measures of 80%. This further supports the longitudinal trend of higher insecticidal effect downstream. Pre treatment and post treatment measures are illustrated in Figure 8.

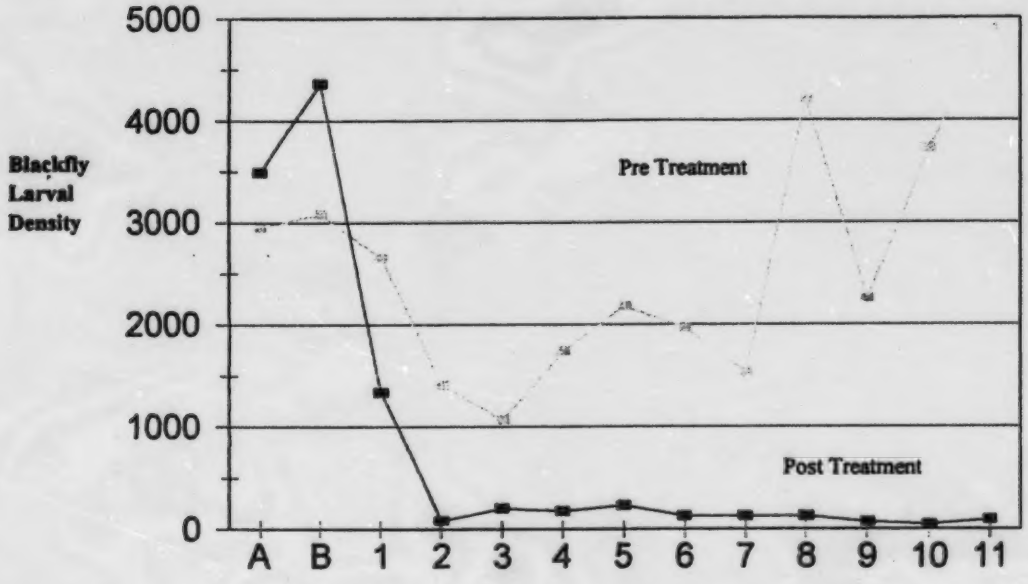


Figure 8. Mean blackfly larval densities observed at 11 exposure stations located equidistantly along 33km study reach of North Saskatchewan River, and; A + B - unexposed reference location upstream of treatment area. Observations collected from artificial substrates before and after treatment with 2.0ppm *B.t.i.*, June 13, 2000. Means were calculated from 3 mortality measures from substrates at 3 depths (n=3, N=39).

4.1.4 2.5ppm

The efficacy trial investigating 2.5 ppm *B.t.i.* was conducted on June 29, 2001. The artificial substrates were placed within the study area 5 days prior to application of *B.t.i.* at the same locations utilized in the previous year. At the time of treatment, the North Saskatchewan water discharge was 275 m^3/s and water temperature was 15°C. Five volumes of 682 litres were applied over 50km for a total of 3410 litres. All artificial substrates were collected before and after treatment including the population drift estimate for a complete estimate of the effect of 2.5 ppm *B.t.i.*

The mean index of mortality for 2.5 ppm *B.t.i.* was calculated to be 0.843 or, 84% of the observed larval density. The standard deviation for this trial was 0.19. Observed larval densities before and after the 2.5 ppm trial are illustrated in Figure 9. The estimate for numbers of blackfly larvae at drift during the application were consistent with previous trials ranging from 228 to zero per meter of substrate. One factor which decreased the index for 2.5ppm mortality was several artificial substrates were scoured of larvae from river sand and sediments. This resulted in several low indices of mortality and in one instance zero mortality was calculated (station 2). The results of this trial were inconsistent with results observed with previous trials in that an increase in *B.t.i.* concentration appeared not to achieve greater blackfly larval mortality. The 2.5ppm trial was consistent in that higher mortalities were observed at downstream locations of the study zone. This further supports the possibility of a cumulative longitudinal effect for *B.t.i.* in these trials.

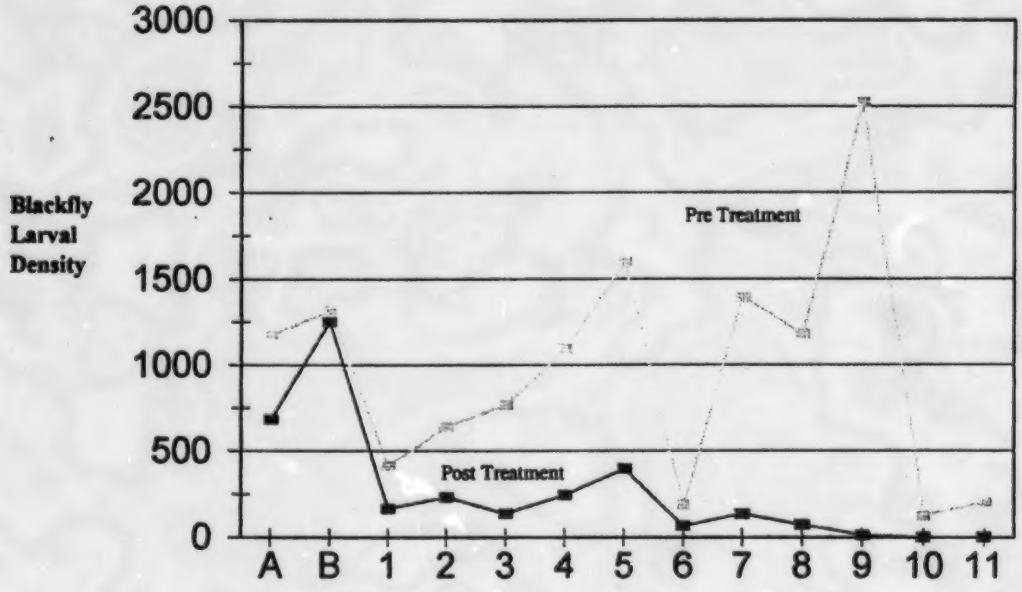


Figure 9. Mean blackfly larval densities observed at 11 exposure stations located equidistantly along 33km study reach of North Saskatchewan River, and; A + B - unexposed reference location upstream of treatment area. Observations collected from artificial substrates before and after treatment with 2.5ppm *B.t.i.*, June 29, 2001. Means were calculated from 3 mortality measures from substrates at 3 depths (n=3, N=39).

4.2 Blackfly Larval Mortality and Comparison of Treatments

The comparison of treatments presented here are based on a relative measure of blackfly larval mortality (Section 3.4.1). Quantified mortality (*ie.* LD50, LD90) is a practical concern for pesticide applicators who wish to achieve a predictable effect on target pest populations. If pest managers can accurately measure blackfly larval populations and recognize the economic threshold of pest populations, the doses required to maintain larval densities below threshold can be determined with greater precision. Optimization of *B.t.i.* applications in this manner improves the programs cost efficiency as well as minimizing pesticide residues in the environment and impact to non target organisms.

Lower mortality for 2.5ppm than 2.0ppm *B.t.i.* trial caused the relationship between *B.t.i.* concentration and blackfly larval mortality to appear asymptotic at higher concentrations. This relationship is illustrated in Figure 10. Although there the relationship appears nonlinear, results of these efficacy trials suggest the LD90 of *B.t.i.* (lethal dose for 90% of population) to be 2.2 ppm with respect to North Saskatchewan River discharge for15min for spring populations of Simuliidae when applied over the entire target area. These results are consistent with previous investigations using the *B.t.i.* product Vectobac 1200L ® in which asymptosis was not as prevalent (Lipsit 2001). The 95% confidence interval for this estimate is 1.4ppm - 3.0ppm which overlaps other treatment application rates. It is therefore meaningful to test if there are significant differences in measured mortality among treatments and if so, identify which *B.t.i.* application rates were different. Since the frequency distributions of measured mortality for each trial demonstrated nonparametric qualities (bimodality and heteroscedasticity) and we hypothesized an ordered response in blackfly mortality to *B.t.i.* concentration, the Page Test for ordered alternatives was appropriate. All samples had been successfully recovered and this provided a full data matrix consisting of $k = 4$ columns (treatments) and $N = 33$ rows for measures of mortality.

The L statistic from the sum of ranks was converted to a Z (Z_L) statistic for large samples and compared with the critical value of the standard normal distribution (Siegal and Castellan 1988). The result was $Z_L = 5.91$ which is larger than $Z_{critical} = 1.6$, $\alpha = 0.05$ and the null hypothesis was

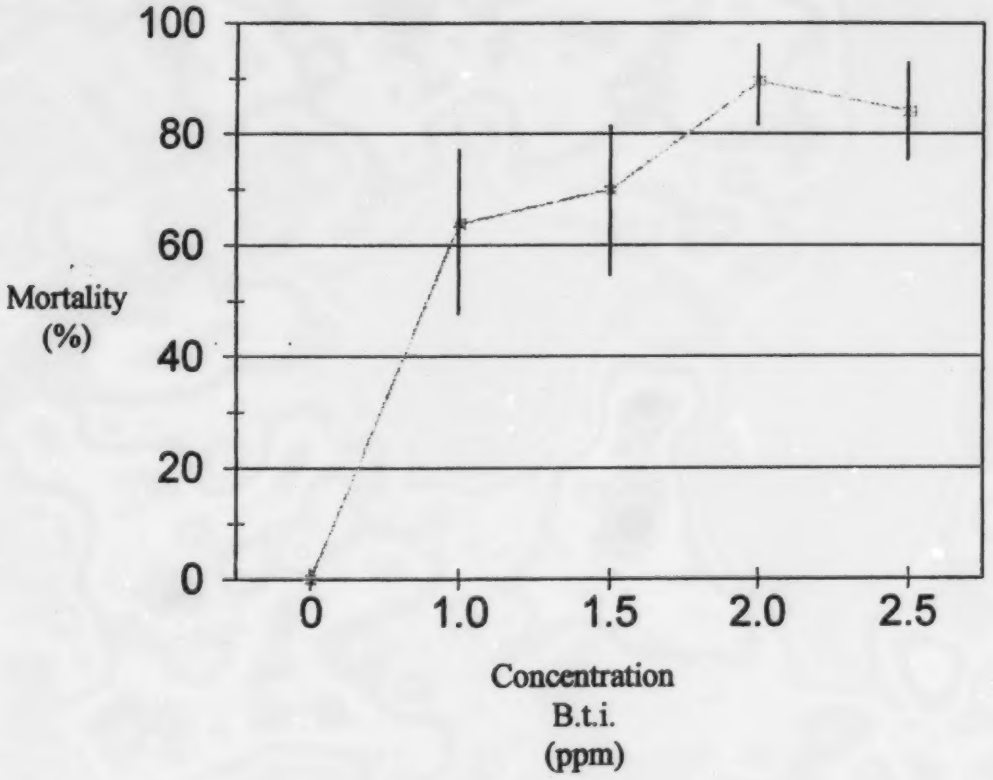
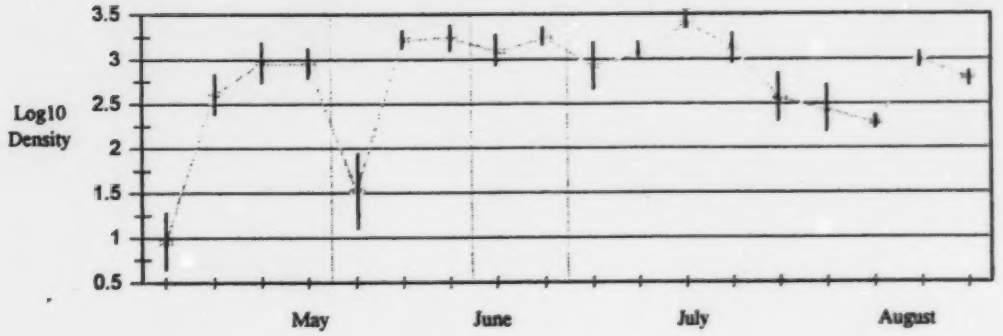
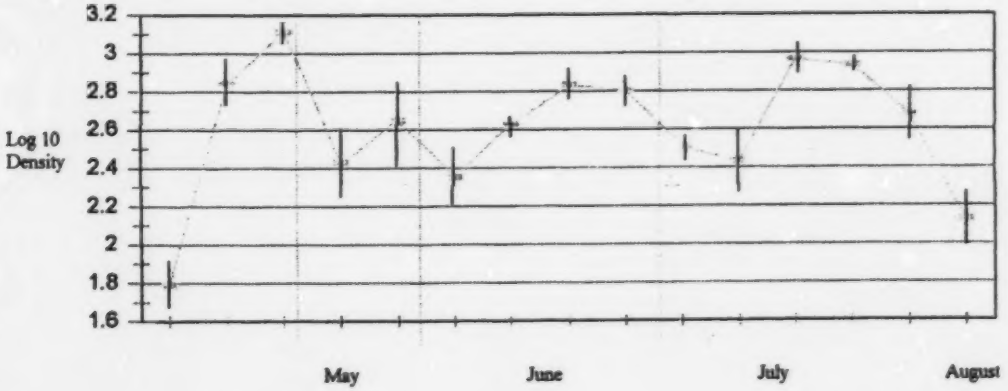


Figure 10. Observed measures of mean blackfly larval mortality from polyethylene artificial substrates collected pre and post treatment of *Bacillus thuringiensis* variety *israelensis* applications upon the North Saskatchewan River. Vertical lines represent standard errors.



2000



2001

Figure 11. Mean abundance (Log (Y+1)) of preimaginal Simuliidae observed on artificial substrates collected from North Saskatchewan River, 2000 and 2001. Vertical bars represent standard errors. Vertical dotted lines represent *B.t.i.* applications to area. Upper illustration = observations in year 2000, lower illustration = 2001.

rejected. We may therefore conclude there are significant differences in the median index of blackfly larval mortality colonizing artificial substrates in the North Saskatchewan River among *B.t.i.* treatment concentrations ($P < 0.0003$). This analysis is summarized in Appendix 1 of this report.

Rejection of the null hypothesis justified multiple comparison between the treatments. The examination compares the difference between 2 sums of ranks with a critical value and identifies which treatment differed significantly from another (Siegal and Castellan 1988). The null hypothesis (H_o) tested is: 'the median index of mortality observed from one treatment is not significantly different from another' and each treatment is compared with all others. As with the Page test of ordered alternatives, multiple comparisons remain a one tailed test with $\alpha = 0.05$ for significant differences to be determined. The critical value for rejection of H_o was 25.1 and 4 of the 6 differences in the sum of ranks were greater than this value in the comparisons. H_o could not be rejected in the comparison of 1.5 ppm *B.t.i.* and 1.0 ppm *B.t.i.* nor could it be rejected in the 2.5ppm and 2.0ppm comparison. However, the treatments of 2.5ppm and 2.0ppm *B.t.i.* were significantly different from the treatments of 1.0 and 1.5ppm and H_o could be rejected at the $\alpha = 0.05$ level and justify the biological conclusion that: 'median blackfly mortality from 2.5ppm and 2.0 ppm *B.t.i.* is greater than the median mortality observed from concentrations of 1.5 ppm and 1.0 ppm *B.t.i.* ($P < 0.05$) from observations of polyethylene artificial substrates.

5. DISCUSSION

5.1 Factors Affecting *B.t.i.* in Field Trials

Comparison of the efficacy trials was intended to resolve the precise effect *B.t.i.* concentration had upon the preliminary spring blackfly population in the North Saskatchewan River. The underlying assumption in the comparison was that *B.t.i.* concentration is the only variable which differed among treatments and other parameters did not affect the observed variation between treatments. Natural field conditions rarely conform to this assumption and although methodology remained consistent, two variables should be considered. River discharges ranged from 180 to 455 m^3/s and water temperatures ranged from 12°C - 16°C at times of application (Table 1).

River discharge influences the drift rates of the blackfly population as well as the seston load characteristics of the river. The relationship between blackfly larval drift and river discharge is positively correlated and a curvilinear function for three species, *S. luggeri*, *S. meridionale*, and *S. vittatum* complex, in the North Saskatchewan River (Jarvis 1987). This relationship is primarily the effect of water velocity upon the constant drift rate of blackfly larval populations or the rate at which individuals enter the water column and are transported downstream (Allan 1987, Reisen 1977). Jarvis (1987) also observed a behavioral drift response of *S. luggeri* and *S. meridionale* which was negatively correlated to changes in river discharge. That is, drift abundance of these two species increased in response to decreasing river discharge, presumably to avoid stranding and dessication as the river littoral area changed. Results from Zhang *et al.* (1998) indicate changes in water flow (hydraulic disturbance) dramatically alter the abundance and species richness of Simuliidae in temperate ecosystems. Therefore, the effect of varying river discharges upon blackfly larval abundance observed in this study is a difficult evaluation.

Although artificial substrates are less susceptible to measurable changes in abundance than are drift net samples (Jarvis 1987) and substrates were placed in midstream to reduce the influence of discharge fluctuations, the sampling approach in this study may be over simplistic. Drift net

samples as well as artificial and natural substrates would provide a more comprehensive examination of *S. luggeri* population dynamics in the Saskatchewan River. Further, collections from mainstream and littoral habitat would undoubtably have been more informative with respect to spatial mortality in the river (Green 1979) but was beyond the scope of this project. North Saskatchewan River discharges were relatively stable at times of *B.t.i.* applications ($\Delta < 5$ m^3/s per day) except for the June 21st trial at 2.0ppm where discharge was increasing more dramatically (10 $m^3/s < \Delta < 5$ m^3/s per day). One aspect of the management strategy of the Saskatchewan control program is to delay *B.t.i.* applications until minimal river discharges are observed to reduce the *B.t.i.* volume required. A more ecumenical strategy would include an evaluation of the stability of discharge to avoid the behavioral drift response of *S. luggeri* and *S. meridionale*.

A further consideration of the varying river discharge is the seston load which is carried downstream. This has been of importance because current velocity and turbidity of the stream largely accounted for the downstream transport of *B.t.i.*, or lack of it, and hence the efficacy observed in early field trials (Undeen and Colbo 1980, Undeen and Lacey 1982). *B.t.i.* and associated toxins are particulate and thus vulnerable settling from the water column depending upon size, mass, other particulates, and the tractive forces of the river. The application method of actively delivering the larvicide to downstream habitat rather than passive delivery by river transport greatly reduces this concern. Increased transport of sediments, which would occur at higher discharge rates, have been implicated in increasing the drift (catastrophic and behavioral) of other aquatic insects (Culp *et al.* 1986) and was also observed for larval Simuliidae (Zhang *et al.* 1998). A concern for *B.t.i.* applicators is that blackfly larvae may be drifting and not ingesting *B.t.i.* particles. Conversely, blackfly rates of feeding during the larval phase have been positively correlated (asymptotically) with seston concentrations and could also affect larvicidal activity of *B.t.i.* (Morin *et al.* 1988). Morin *et al.* (1988) demonstrated with mesocosms (gutter systems) that *S. venustum verecundum* complex normally feeds at rates close to maximum under natural seston loads (> 30mg/l) and limited ingestion rates would occur at greatly reduced seston concentrations (1-2 mg/l). Ingestion rates varied significantly among 7 blackfly species with respect to seston concentration in the Morin *et al.* (1988) study but the response curves for each species were similar in shape suggesting the relationship between

seston concentration and ingestion rates to be common among blackfly larvae. Fredeen (1983) reported turbidity in the North Saskatchewan River to range from 10 - 1000 mg/l approximately with discharge rates. Although caution must be exercised in interspecific interpolations, it seems unlikely that seston concentration contributed greatly to variance observed in the Saskatchewan River trials. Gross changes in turbidity or discharge rates were not observed during these trials.

Another variable of the North Saskatchewan efficacy trials which differed during trials was water temperature. Similar to seston concentration, temperature does not directly affect the *Bacillus thuringiensis* toxins but rather influences the ingestion rates of blackfly larvae which indirectly affects larvicidal activity. Morin *et al.* (1988) reports ingestion rates of *S. venustum/verecundum* complex increase exponentially with increases in temperatures between 9-19°C. He also argues the relationship would not vary greatly among species although the relative change in feeding rate must be described for each species. These data would suggest a given dose of *B.t.i.* would have higher mortality at warmer temperatures given toxicity is dependant upon amount ingested. In these trials the highest mortality coincided with higher *B.t.i.* concentration (2.0 ppm) and the highest observed temperature (15°C and 16°C). The lowest mortality (63%) occurred at 15°C with 1.0ppm *B.t.i.* The observed similarity in mortality measures between 1.0 and 1.5ppm may be a function of the dissimilarity in water temperatures (10°C - 15°C).

Measured mortality proved to be curvilinear with respect to *B.t.i.* concentrations although asymptotic at the upper end of mortality as would be expected (Figure 10). It is possible that the observed range of temperatures during the trials do not lie within a sensitive region of the *S. luggeri* temperature/feeding response curve and hence greater differences were not detected. It is also reasonable to assume temperature would influence observed blackfly mortality greater at lower *B.t.i.* concentrations because fewer toxins would be available within the entire seston load. The precise effect of temperature upon ingestion rates of economically important blackflies in Saskatchewan should be further investigated in the laboratory. Temperature could not be controlled in this scale of field trial, nor can it be discounted as a source of error.

5.2 Study Implications for the Saskatchewan Blackfly Control Program

In each application of *B.t.i.* to blackfly larval habitat a measurable reduction of blackfly abundance was observed upon artificial substrate populations. The reduction in abundance was positively correlated with *B.t.i.* concentration within the river discharge and may be modified by river conditions and the dynamics of the blackfly population. In this section the predictability of the reduced abundance, reliability of the measure, and factors which should be considered to maximize the effect of *B.t.i.* upon the target population are discussed.

5.2.1 General Observations of Effect of *B.t.i.* Upon Larval Simuliidae

The observations used to describe treatment effect were an average of spatially distributed mortality measures obtained from artificial substrates. Higher mortality measures were observed at downstream locations in every trial, indicating a cumulative effect of successive upstream applications of *B.t.i.* The skewness of mortality frequency distributions observed in this study were evidence of lower, mortality observations from upstream locations. Although the frequency distributions in each trial were asymmetrical, it should be noted this does not preclude a non-normal distribution (Snedecor and Cochran 1989). The evidence of a cumulative downstream effect observed in these trials and in earlier work (Agriculture Development Fund 1990) supports Lehmkuhl's (1990) recommendation that the Saskatchewan blackfly program take a complete methodology to river treatments and population monitoring. That is, a 10 km section of river should not be treated because of high blackfly abundance observed from one sampling location. Rather, the river should be treated in its entirety when all sampling locations indicate larval abundance is beyond economic thresholds. Results of these field trials indicate lower *B.t.i.* rates may be used for downstream areas while using greater rates upstream during complete river applications. Considerable cost savings can be realized from this strategy alone.

A further aspect of the spatial effect of *B.t.i.* which was not examined in this study is comparative reductions in larvae occupying littoral habitat and mainstream locations. In this study all artificial substrates were collected from mainstream sampling locations. A more

comprehensive study design would include sampling locations in shallows near the river banks. Mason and Kusters (1990b) suggest the Saskatchewan application procedure would maximize the effect of *B.t.i.* by applying 60-70% of the larvicide in the mainstream and less larvicide to the littoral areas since greater water volumes and current velocity occur in the main channel. Professional *B.t.i.* applicators from Sylvico Inc. (1999) recommend the opposite proportions with the logic that aquatic macrophytes are more abundant at the banks and hence more larvae will occur there. This must certainly be described for each branch of the Saskatchewan River due to differences in their respective profiles, particularly the distribution of vegetation. Surveys of each river or branch could clarify the issue of the spatial distribution of blackfly larvae. Presently the application technique utilized by the Saskatchewan blackfly control program should provide effective distribution of *B.t.i.* The downstream delivery of *B.t.i.*, navigation in a 'zig-zag' pattern from bank to bank, treatment of the entire river, and mixing from the river hydro-dynamics as the *B.t.i.* moves downstream undoubtably result in the maximum coverage of the habitat.

Further considerations for the optimization of *B.t.i.* applications to the Saskatchewan River involve the timing of treatments. Two factors were identified in this study which had the greatest influence upon observed blackfly mortality. The primary factor was *B.t.i.* concentration applied, and the second was the proportion of the blackfly population at drift during treatment. The constant drift of *S. luggeri* or other target blackflies is unavoidable at time of treatment. However, recognizing stream or river conditions which induce a behavioral drift response or catastrophic drift among blackflies and avoiding these conditions are critical for the optimization of *B.t.i.* Drift density from a behavioral response of *S. luggeri* was positively correlated with decreasing river discharges and drift abundance was correlated with higher discharges (Jarvis 1987). Catastrophic drift of Simuliidae and other aquatic macroinvertebrates occurs during major disturbances of the aquatic habitat (*e.g.* pesticide applications or drastic change in current velocity and seston load; Crosskey 1990, Dosdall and Lehmkuhl 1989b, Jarvis 1987). Therefore, during times of constant discharge, a stable, sessile blackfly population exists which would optimize *B.t.i.* treatments. Of course, larvicide savings will be realized at minimal river discharges if these can be accurately predicted.

A final consideration for optimization of *B.t.i.* identified in the literature is that of temperature. Although unsupported by observed mortality levels in this study, temperature has been demonstrated to influence the ingestion rates of simulid larvae (Crosskey 1990, Morin *et al.* 1988) and hence the ingestion of *B.t.i.* toxins. The Saskatchewan blackfly control program, whenever possible, should time *B.t.i.* applications during periods of stable, minimal discharge and maximum water temperatures. This observation should include diel fluctuations in temperature should they occur during the daily photo period.

5.2.2 Precision and Reliability of *B.t.i.* Dosages

This study has demonstrated *B.t.i.* to reduce blackfly abundance upon artificial substrates in a curvilinear fashion which is positively correlated with *B.t.i.* concentration within the water column. The relationship of *B.t.i.* dosages and reduction of blackfly density upon artificial substrates, the calculated LD90, and the standard errors associated with trials in the North Saskatchewan River have been described. It is therefore theoretically possible to determine precise *B.t.i.* dosages which are required to effectively reduce blackfly larvae below a desired threshold. The calculation is a relatively simple one if 3 criteria are established *a priori*. These criteria are:

1. Identifying the adult blackfly density of economic importance within the area to be controlled.. Normally this measure can be established by quantifying the economic injury to man or property, the cost of implementing control measures, and balancing an effective cost - benefit ratio (Johansen 1985, Gillott 1980). These thresholds are often established intuitively such as zero tolerance in the case of sensitive vector control programs, or quantitatively using landing/biting counts in cases of more subjective tolerance levels for 'annoyance' populations.

2. Specific blackfly larval density values must be identified which generate adult densities of economic magnitude. Adult pest population densities must be correlated with the larval densities from the aquatic source if larvicide strategies are to maintain adult population levels. Monitoring larval populations in conjunction with economic impact

assessments would be the most informative type of correlation (Fredeen 1985a).

3. The blackfly larval sampling regime must be quantified with the true standing crop of aquatic Simuliidae. Does the sampling method accurately reflect natural population densities or does the sample require correction to become more precise?

Fredeen (1985a) had assessed the economic losses to central Saskatchewan livestock producers as high as $3,000,000.^{00}$ (Can.) during moderate to severe infestations of *S. luggeri*. The Saskatchewan blackfly control program currently operates within a budget of $125,000.^{00}$ (Can.) per annum. Qualitative assessments from producer cooperatives suggest the program has been successful in avoiding outbreaks of the magnitude described by Fredeen (1985a) and hence the program could be considered profitable in terms of applied entomology (Gillott 1980). There has been no study since Fredeen's (1985a) evaluation as to the economic impact of *S. luggeri* and therefore the success of the Saskatchewan control program remains qualitative. The Saskatchewan control program currently monitors adult field populations of blackflies using silhouette traps established at 7 pastures within the designated control zone. Although quantitative replicates are collected, they are presently only meaningful in comparisons between annual mean densities or relative success of the control program. Additional economic assessments of blackfly impact similar to the Fredeen (1985a) study, concurrent with silhouette trap collections, are required to clarify Saskatchewan blackfly densities in economic terms. Until then the quantitative success of the program will remain obscure.

The precision with which the Saskatchewan blackfly program wishes to reduce blackfly larval densities with respect to the economic savings (both in larvicide and livestock production) depends on the accuracy of larval density estimates and their relationship with adult pest populations. Fredeen (1985a) monitored larval densities with estimates from artificial substrates during his study of the economic impact of *S. luggeri* in Saskatchewan and did not report quantitatively the adult population infesting the study area. Although establishing a direct correlation between artificial substrate estimates of larval populations and economic impact of emergent adults, the economic threshold for larval densities remains an intuitive one (Mason and Kusters 1990b). Maintenance of blackfly larval densities upon polyethylene tapes below 1000

larvae undoubtably avoided economic impacts similar to those described by Fredeen (1985a) in the experience of program managers (Fredeen 1975, 1985a, Mason and Kusters 1990a, 1990b). If minimal dosages are a to be a tenet of the program, further research is required describing negligible or moderate infestations of blackflies and the associated larval densities observed upon polyethylene tape substrates. That is, the quantitative relationship between larval densities and adult pest populations should be described more comprehensively.

The third criteria we have described addresses the larval sampling methodology where drift colonization of polyethylene substrates is the measure of the blackfly standing crop density. Polyethylene tapes are perhaps an appropriate sampling method for many areas of a large river system in terms of cost effectiveness and sample efficiency (Fredeen and Spur 1978, Merritt *et al.* 1989). They are not a direct measure of blackfly abundance however, and inferences concerning economic thresholds from these observations should be made cautiously (Resh and McElvary 1993). To improve the precision of larvicide applications and reducing blackfly abundance, it is critical that the relationship between larval sample and standing crop be understood. For example, the observation of 1000 larvae per meter of artificial substrate to represents substantially different values of true blackfly abundance in the North Saskatchewan and South Saskatchewan Rivers. If 1m of artificial substrate represents 1m of aquatic macrophyte (in the case of *S. luggeri*), then macrophyte density is certainly a variable in the expression of standing blackfly abundance for a given reach of river.

This point is discussed due to gross differences in the profile of the North and South Saskatchewan Rivers. Due to shallow, clear water in the South Saskatchewan River, vegetation is abundant and somewhat uniform throughout the river in the study zone whereas the North Saskatchewan turbidity limits growth of aquatic macrophytes to small patches of *Potamogeton crispus* along banks and back eddies. Zhang *et al.* (1998) also report significant differences in blackfly abundance and species composition between regulated and unregulated watersheds. Therefore, it is further recommended the Saskatchewan blackfly control program verify the relationship of blackfly densities occurring upon polyethylene substrate samples and the natural abundance and distribution of blackfly larvae within the study reach.

6. SUMMARY

The control of blackfly larvae in their lotic environment is somewhat unique to conventional pest management strategies. Blackfly larvae are normally the sole target of a very complex community within an atypical pest environment. The effects of organophosphates and organochlorines cause catastrophic changes (*i.e.* mortality) in the aquatic community and drift of the benthic assemblage (Hurlbert *et al.* 1971, Wallace and Hynes 1975, Dosdall and Lehmkuhl 1989a, 1989b). In addition to the nontarget impact, there is the reality of insect resistance to synthetic chemicals currently applied (Curtis *et al.* 1993, Ritchie 1994). If we accept the tenet of minimal impact to the nontarget community in pest control efforts, biological controls are an attractive alternative. In fact, what many consider the primary drawback of biological controls in general (that they are too specific) is desirable in blackfly applications. Some cases of crop protection may require general pesticides which are effective to pests of several insect orders, whereas a pesticide with the specificity of *B.t.i.* is preferable in lotic applications to minimize nontarget impact and maintain comparatively healthier food webs.

Innundation with *B.t.i.* is not a biological control which maintains simuliid populations at stable equilibrium below Saskatchewan agro-economic levels. *B.t.i.* is however, a bioinsecticide capable of significant reduction of blackfly larval populations as well as demonstrating high specificity and minimal residues. This may be the best solution possible for an insect pest with an ephemeral natural history and capable of rapid population increases (Gillott 1980). In general, *B.t.i.* treatments which reduced *S. luggeri* abundance below 1000 larvae per meter of substrate effectively maintained this level for a 2 week period before drift and recruitment re-established economically significant populations.

It was intended these results provide a more definitive framework in the decision making process of *B.t.i.* application rates for optimizing cost effectiveness in the Saskatchewan blackfly control program. The exercise to date has demonstrated predictable reduction of blackfly populations colonizing artificial substrates in mainstream river habitat. The precision of the reduction can be modified by environmental factors (*ie.* water temperature) and may have been expressed in these results. The trials have indicated *B.t.i.* applications may be optimized in three

ways:

1. Reduce *B.t.i.* concentrations incrementally downstream due to an apparent cumulative longitudinal effect;

2. When only a moderate mortality is required to reduce blackfly densities below agro-economic thresholds 1.0ppm *B.t.i.* can be used as effectively as 1.5ppm (no significant difference in observed mortality). Similarly 2.5ppm *B.t.i.* did not achieve significantly greater mortality than 2.0ppm *B.t.i.* therefore 2.0ppm concentrations should be used for greatest results;

3. Timing treatments whenever possible with low, stable river discharges and maximum water temperatures.

This study also described the necessity of quantifying the relationship between adult field populations of pestiferous blackflies and the benthic standing crop in aquatic habitat as estimated from a sampling regime. Rigorous sampling of the blackfly larval population is required for an accurate estimate of the standing crop but just as important is the recognition of economic thresholds of larval densities as they will relate to adult field populations. When these criteria have been met *B.t.i.* applicators can objectively determine a minimal *B.t.i.* dosage required to reduce blackfly larval abundance below the threshold thereby increasing cost effectiveness and minimizing pesticide impacts to non-target organisms or residues. Further research will reduce the observed variance in mortality when the factors of discharge, temperature and seston concentrations have been described for regional conditions and simuliid species.

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APPENDIX 1. Comparative summary of *B.t.i.* larvicide treatments on blackfly abundance observed from artificial substrates in North Saskatchewan River, 1995 - 1999.

A. Summary of observed reduction of blackfly larvae upon artificial substrates within *B.t.i.* exposure zone and their associated ranks with respect to other treatments.

Location	Depth	2.5ppm Mortality	Rank	2.0ppm Mortality	Rank	1.5ppm Mortality	Rank	1.0ppm Mortality	Rank
	1 - 2m	0.70	1	0.56	2	0.00	4	0.40	3
Station 1	3 - 4m	0.80	1	0.50	3	0.63	2	0.00	4
	5 - 6m	0.78	2	0.59	3	0.55	4	0.99	1
	1 - 2m	0.8	2	0.95	1	0.78	3	0.47	4
Station 2	3 - 4m	0.74	3	0.94	1	0.77	2	0.55	4
	5 - 6m	0	4	0.93	2	0.98	1	0.00	3
	1 - 2m	0.74	2	0.86	1	0.03	4	0.51	3
Station 3	3 - 4m	0.84	1	0.81	2	0.00	4	0.43	3
	5 - 6m	1.0	1	0.83	2	0.45	3	0.09	4
	1 - 2m	0.76	3	0.80	2	0.86	1	0.71	4
Station 4	3 - 4m	0.68	3	0.93	1	0.85	2	0.00	4
	5 - 6m	0.92	2	0.92	1	0.76	3	0.24	4
	1 - 2m	0.85	2	0.92	1	0.76	3	0.52	4
Station 5	3 - 4m	0.72	3	0.90	1	0.85	2	0.57	4
	5 - 6m	0.71	3	0.87	1	0.75	2	0.43	4
	1 - 2m	0.83	3	0.95	1	0.82	4	0.90	2
Station 6	3 - 4m	0.59	4	0.95	1	0.71	3	0.75	2
	5 - 6m	0.76	2	0.92	1	0.63	4	0.72	3
	1 - 2m	0.91	1	0.82	2	0.32	4	0.79	3
Station 7	3 - 4m	0.93	1	0.92	2	0.78	4	0.91	3
	5 - 6m	0.88	3	0.96	1	0.70	4	0.90	2
	1 - 2m	0.92	2	0.97	1	0.80	4	0.83	3
Station 8	3 - 4m	0.96	2	0.97	1	0.84	3	0.78	4
	5 - 6m	1.0	1	0.97	2	0.78	3	0.78	4
	1 - 2m	0.98	1	0.98	2	0.96	3	0.90	4
Station 9	3 - 4m	1.0	1	0.96	2	0.28	4	0.89	3
	5 - 6m	1.0	1	0.97	3	0.81	4	0.98	2
	1 - 2m	1.0	1	0.98	2	0.94	4	0.95	3
Station 10	3 - 4m	1.0	1	0.99	2	0.96	3	0.50	4
	5 - 6m	1.0	1	0.99	2	0.97	3	0.87	4
	1 - 2m	1.0	1	0.98	2	0.93	3	0.91	4
Station 11	3 - 4m	1.0	1	0.99	2	0.93	3	0.86	4
	5 - 6m	1.0	1	0.98	2	0.93	4	0.95	3
	Sum		61		55		104		110

A. Calculation of L statistic for Page Test of Ordered Alternatives

$$L = 61 + 2(55) + 3(104) + 4(110)$$

$$\mathbf{L = 923}$$
$$N = 33$$
$$K = 4$$

C. Conversion of L statistic to Z_L for comparison with critical value from Student's Normal distribution (Siegal and Castellan 1988).

$$z_L = [12(L) - 3Nk\,(k + 1)^2 / k\,(k^2 - 1)]\,[(k - 1) / N]1/2$$
$$z_L = [12(923) - 3(33)4(4 + 1)^2 / 4\,(4^2 - 1)]\,[(4 - 1) / 33]^{1/2}$$
$$z_L = [(11076 - 9900)/60][0.302]$$

$$\mathbf{z_L = 5.91}$$

B. Multiple Comparisons among treatments. $Z_{(0.05)/k(k-1)} = 2.128$ (Siegal and Castellan 1988).

$$Z_{(0.05)/k(k-1)} = 2.394$$
$$\text{Critical value} = 2.394\,(N\,k\,(k+1)/6)^{-1}$$
$$\text{Critical value} = 2.394\,(33*4(4+1)/6)^{-1}$$
$$\text{Critical Value} = 25.11$$

$$R_{2.5\ ppm} - R_{2.0\ ppm} = 61 - 55 = 6$$
$$R_{2.5\ ppm} - R_{1.5\ ppm} = 61 - 104 = \mathbf{43}\ *$$
$$R_{2.5\ ppm} - R_{1.0\ ppm} = 61 - 110 = \mathbf{49}\ *$$
$$R_{2.0\ ppm} - R_{1.5\ ppm} = 55 - 104 = \mathbf{49}\ *$$
$$R_{2.0\ ppm} - R_{1.0\ ppm} = 55 - 110 = \mathbf{55}\ *$$
$$R_{1.5\ ppm} - R_{1.0\ ppm} = 104 - 110 = 6$$

* Significant $P < 0.05$, $Z_{(0.05)/k(k-1)} = 2.394$, $N = 30$, $k = 3$

APPENDIX 2. QA/QC results of 10 sort checks on blackfly artificial substrate samples from the North Saskatchewan River trial 1.5ppm May 13, 2000.

Station	Sample	Sort Check
Reference A	Tape #3	0 blackflies
Reference B	Tape #6	0 blackflies
Exposure #2	Tape #2	0 blackflies
Exposure #3	Drift #3	0 blackflies
Exposure #4	Tape #5	0 blackflies
Exposure #6	Tape #2	0 blackflies
Exposure #7	Tape #4	0 blackflies
Exposure #8	Tape #6	0 blackflies
Exposure #9	Tape #1	0 blackflies
Exposure #11	Tape #4	0 blackflies